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Drinking Water and Health

Part I
Chapters 1-5

The National Research Council

A Report of the Safe Drinking Water Committee

Advisory Center on Toxicology

Assembly of Life Sciences

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Drinking Water and Health

Part I
Chapters 1-5

A Report of the Safe Drinking Water Committee
Advisory Center on Toxicology Assembly of Life Sciences
National Research Council

NATIONAL ACADEMY OF SCIENCES
Washington, D. C. 1977

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the Councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the Committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, The National Academy of Engineering, and the Institute of Medicine.

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DRINKING WATER AND HEALTH

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PREFACE

This report is a study of the potentially harmful effects that impurities in drinking water may have on human health. The study was conducted by the Safe Drinking Water Committee of the National Research Council, supported by a contract between the Environmental Protection Agency (EPA) and the National Academy of Sciences.

The Safe Drinking Water Act of 1974 required the Administrator of the EPA to make arrangements for this study so that the results might serve as the scientific basis for revising the interim primary drinking water regulations that were promulgated under the Act.

Our ability to respond to the specific terms of the Act was limited by the present state of scientific knowledge. Recognizing this limitation, the study was conducted in accordance with the interpretation given in Appendix A.

Application of advanced methods of analysis has widened our knowledge of the trace impurities of water much faster than the rate of accumulation of information about their toxicity. Consequently, as new information becomes available, further investigation will be required to estimate the health effects of water that could not be assessed in this study.

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Gerard A. Rohlich, Chairman

Safe Drinking Water Committee

Historical Note

As noted by Baker (1949), the quest for pure water began in prehistoric times. Recorded knowledge of water treatment is found in Sanskrit medical lore and in Egyptian inscriptions. Pictures of apparatus to clarify liquids, (both water and wine), have been found on Egyptian walls dating back to the fifteenth century B.C. Boiling of water, the use of wick siphons, filtration through porous vessels, and even filtration with sand and gravel, as means to purify water are methods that have been prescribed for thousands of years. In his writings on public hygiene, Hippocrates (460-354 B.C.) directed attention principally to the importance of water in the maintenance of health, but he also prescribed that rain water should be boiled and strained. The cloth bag that he recommended for straining became known in later times as "Hippocrates' sleeve".

Public water supplies, already developed in ancient times, assumed added importance with the progressive increase in urbanization. But though they were clearly beneficial in distributing water of uniform quality, large numbers of people ran the risk of having adverse effects when the water was unsafe to drink.

The first clear proof that public water supplies could be a source of infection for humans was based on careful epidemiological studies of cholera in the city of London by Dr. John Snow in 1854 (Snow, 1855). Although Snow's study of the contaminated Broad Street pump is the most famous, his definitive work concerned the spread of cholera through water supplied by the Southwark and Vauxhall Company and the Lambeth Company. The former obtained its water from the Thames at Battersea, in the middle of London in an area almost certainly polluted with sewage, whereas the Lambeth Company obtained its water considerably upstream on the Thames, above the major sources of pollution. In one particular area served by these two companies, containing about 300,000 residents, the pipes of both companies were laid in the streets, and houses were connected to one or the other sources of supply. Snow's examination of the statistics of cholera deaths gave striking results. Those houses served by the Lambeth Company had a low incidence of cholera, lower than the average population of London as a whole, whereas those served by the Southwark and Vauxhall Company had a very high incidence. As the socioeconomic conditions, climate, soil, and all other factors were identical for the populations served by the two companies, Snow concluded that the water supply was transmitting the cholera agent. Snow's study, a classic in the field of epidemiology, is even more impressive when it is realised that at the time he was working, the germ theory of disease had not yet been established.

During the 17th to the early 19th century a number of improvements in water supply were made, most of these related to improvements in filtration to remove the turbidity of waters. During this same period, the germ theory of disease became firmly established as a result of research by Louis Pasteur, Robert Koch, and others, and in 1884 Koch isolated the casual agent of cholera, *Vibrio cholera*.

Importance of water filtration. In 1892, a study of cholera by Koch in the German cities of Hamburg and Altona provided some of the best evidence of the importance of water filtration for protection against this disease (Koch, 1894). The cities of Hamburg and Altona both received their drinking water from the Elbe River, but Altona used filtration, since its water was taken from the Elbe below the city of Hamburg and hence was more grossly contaminated. Hamburg and Altona are contiguous cities, and in some places the border between the two follows a contorted course. Koch traced the incidence of cholera in the 1892 epidemic through these two cities, with special attention directed to the contiguous areas. In such areas it was assumed that climate, soil, and other factors would be identical, the principal variable being the source of water. The results of this study were clear-cut: Altona, even with an inferior water source, had a markedly lower incidence of cholera than Hamburg. Since by this time it was well established that cholera was caused by intestinal bacteria excreted in large [illegible]s in the feces, it was concluded that the role of filtration was to remove the contaminating bacteria from the water.

In the United States, cholera was not a problem after the mid-19th century; the water-borne disease of particular concern was typhoid fever. In England, William Budd had shown by the mid-nineteenth century that typhoid fever was a contagious disease, and the causal agent was isolated and identified by Eberth in 1880 and Gaffky in 1884 (Wilson and Miles, 1957). Although the causal agent, now called *Salmonella typhi*, is transmitted in a variety of ways, one of the most significant is by drinking water.

Experiments on water filtration were carried out in the United States during the late 1880's and early 1890's, notably by the Massachusetts State Board of Health experiment station established in 1887 at the city of Lawrence. At this station the treatment of water as well as sewage was considered by an interdisciplinary group that included engineers, chemists, and biologists. A leader in this work was W. T. Sedgwick, a professor at the Massachusetts Institute of Technology, and M.I.T.'s influence on water supply research remained strong throughout the first quarter of the twentieth century. Much of the history of this work has been reviewed by Whipple (1921) and in the two editions of Hazen's book (1907, 1914); the technical aspects are discussed and clearly illustrated by Johnson (1913). One important technological advance which made water filtration adaptable to even rather turbid sources of water was the use of chemical-coagulation filtration processes, patented about 1884 by the brothers J. W. and I. S. Hyatt.

While the Lawrence experiments were going on, an epidemic of typhoid swept through the city, hitting especially hard at those parts that were using the Merrimac River as its water supply. As a result, the city of Lawrence built a sand filter, and its use led to a marked reduction in the typhoid fever incidence. As reported by Hazen (1907), the death rate from typhoid fever in Lawrence dropped 79% when the five year periods before and after the introduction of the filter were compared. Of additional interest was a reduction in the general death rate (all causes) of 10%, from 22.4 to 19.9 per 1,000 living.

Another major series of filtration experiments were made in 1895-1997 at Louisville, Kentucky, where the source of water was the muddy and polluted Ohio River. These experiments were successful, and from an engineering point of view were of importance because they showed that it was possible to treat source waters of a rather poor quality (the Merrimac River at Lawrence may have been polluted, but at least it was a clear water, making filtration rather easier.) The success of the Louisville experiments and the other studies led to rapid establishment of filters as a means of water purification; by 1907 Hazen could list 33 cities in the United States, some of comparatively large size, which were using mechanical filters, and 13 cities that were using slow sand filters. As discussed by Hazen, filtration led to an elimination of turbidity and color from the water, and to a removal of about 99% of the bacteria present. At that time these conditions were considered as a standard by which the quality of a treated water should be judged. As Hazen states: "There is no final reason for such standards. They have been adopted by consent because they represent a purification that is reasonably satisfactory and that can be reached at a cost which is not burdensome to those who have to pay for it... There is no evidence that the germs (characteristic of sewage pollution) so left in the water are in any way injurious. Certainly if injurious influence is exercised it is too small to be determined or measured by any methods now at our disposal." This last statement is of considerable importance when considered in the light of the important advance in water purification practice yet to come, chlorination.

An excellent overview of the relationship between water quality and typhoid fever incidence was published at about this time by Fuertes (1897). He gathered typhoid fever statistics for a large number of cities in North America and Europe, and grouped the data by type of source water and water treatment.

Chlorination, the most significant advance in water treatment. Although a reading of Hazen's 1907 book might lead one to conclude that excellent water quality had been well established by filtration, the most important technological advance in water treatment was yet to come. The introduction of chlorination after 1908 provided a cheap, reproducible method of ensuring the bacteriological quality of water. Chlorination has come down to us today as one of the major factors ensuring safety of our drinking water.

Calcium hypochlorite was manufactured industrially for use as a bleaching powder and was used in paper mills and textile industries. It was a cheap chemical, and hence readily adaptable to use on the large scale necessary for drinking water. The first practical demonstration in the United States of its use in water supply was at the filter plant of the Chicago Stock Yards, where it was introduced by Johnson in the fall of 1908 (Johnson, 1913).

The use of chlorination in an urban water supply was introduced in Jersey City, New Jersey, in the latter part of 1908. The circumstances surrounding the Jersey City case are of some interest from a historical point of view and will be briefly reviewed. Jersey City received its water from a private company which used a large reservoir at Boonton, an impoundment of the Rockaway river. The water was supplied to the city unfiltered, although some settling took place in the reservoir. Several years before 1908 the city raised the contention that the water being supplied was not at all times pure and wholesome for drinking, as was required by the terms of its contract with the private company. At certain times of the year, the water in the reservoir became polluted as a result of sewage influx from communities on the river above the reservoir. Rather than undergo the expense of a filtration plant, or attempt to control the sewage influx from the various communities, the private company chose to introduce a chlorination system. The results were dramatic: a marked drop in total bacterial count was obtained, and at a cost far lower than any other procedure. After many months of operation, further testimony before the court was held, to determine whether the company was meeting its contract, and the court decided that the evidence was favorable to the company. As stated by the court examiner: "I do therefore find and report that this device (chlorination) is capable of rendering the water delivered to Jersey City pure and wholesome for the purposes for which it is intended and is effective in removing from the water those dangerous germs which were deemed by the decree to possibly exist therein at certain times."

The dramatic effect that chlorination had on water supply problems is well illustrated by comparing the first and second editions of Hazen's book (1907 and 1914). In the first edition, barely any mention of disinfection is made (merely a remark about ozone being too expensive) but in the second edition, Hazen waxes enthusiastic about the advantages of chlorination. As he says, chlorination could be used "at a cost so low that it could be used in any public waterworks plant where it was required or advantageous... When the advantages to be obtained by this simple and inexpensive treatment became realized, as a result of the publicity given by the Jersey City experience, the use of the process extended with unprecedented rapidity, until at the present (1914) the greater part of the water supplied in cities in the United States is treated in this way or by some substitute and equivalent method."

Interestingly from the point of view of the present report, the introduction of chlorination also changed markedly the established ideas about water quality standards: "The use of methods of disinfection has changed these standards radically. By their use it has been found possible to remove most of the remaining bacteria so that the water supplied can be as easily and certainly held within one-tenth of one percent of those in the raw water, as it formerly could be held within one percent... Even today the limit has not been reached. It may be admitted that the time will come when a still higher degree of bacterial efficiency will be required. Present conditions do not seem to demand it, but we must expect that in some time in the future conditions will arise which will make it necessary. When additional purification is required it can be furnished." (Hazen, 1914).

The importance of Hazen's book is that Hazen was a major consulting engineer for a wide variety of water works, and was very influential in recommending treatment methods. Chlorination was introduced at about the time that adequate methods of bacteriological examination of water had developed, permitting an objective evaluation of the efficiency of treatment. This evaluation was not based on the incidence of typhoid fever directly, but was based on an indirect evaluation using bacterial or coliform counts.

Soon after chlorination was introduced, it was possible to obtain firm epidemiological evidence that cities chlorinating water had lowered incidences of typhoid fever (Whipple, G.C. 1921). Filtration was introduced in 1906 and chlorination in 1913, and both led to marked reductions in the incidence of typhoid fever. Another dramatic example derives from observations at Wheeling, West Virginia in 1917-1918 (Gainey and Lord, 1952). The incidence of typhoid fever in Wheeling was 155-200 per 100,000 during these years. Chlorination was introduced in the latter part of 1918 with the result that during the first three months of 1919 only 7 cases were recorded. For three weeks during April 1919 chlorination was discontinued with the result that the number of cases increased to 71, or a 300% increase. Chlorination was continued thereafter, and only 11 cases were recorded for the last six months of the year. Other examples of this sort could be cited (Gainey and Lord, 1952).

Summary. We thus see that by the beginning of World War I the essential features of water purification techniques were known, and their worth had been well established. Since that time there have been many refinements made at an engineering level, but no changes in the basic concepts. It is clear that the prime motivation for the development and introduction of purification methods has been to protect the public health, with special concern for controlling the spread of typhoid fever. An ancillary consideration has been esthetics, showing concern for the appearance, taste, and odor of the water.

One point worth emphasizing is that the availability of adequate treatment methods has influenced the standards for drinking water. This point was implied in the books by Hazen (1907 and 1914), but is most clearly seen in the preamble to the 1925 Federal Standards, which superseded the brief 1914 Standards (see Standard Methods, 7th edition, 1933, p. 136 for the complete 1925 Standards). The following quote is relevant:

"The first step toward the establishment of standards which will insure the safety of water supplies conforming to them is to agree upon some criterion of safety. This is necessary because 'safety' in water supplies, as they are actually produced, is relative and quantitative, not absolute. Thus, to state that a water supply is 'safe' does not necessarily signify that absolutely no risk is ever incurred in drinking it. What is usually meant, and all that can be asserted from any evidence at hand, is that the danger, if any, is so small that it cannot be discovered by available means of observation. Nevertheless, while it is impossible to demonstrate the absolute safety of a water supply, it is well established that the water supplies of many of our large cities are safe in the sense stated above, since the large populations using them continuously have, in recent years, suffered only a minimal incidence of typhoid fever and other potentially waterborne infections. Whether or not these water supplies have had any part whatsoever in the conveyance of such infections during the period referred to is a question that cannot be answered with full certainty; but the total incidence of the diseases has been so low that even though the water supplies be charged with responsibility for the maximum share which may reasonably be suggested, the risk of infection through them is still very small compared to the ordinary hazards of everyday life."

At present other considerations make it necessary (for us) to be less confident than was the 1925 Committee on Standards.

Typhoid fever and cholera are dramatic diseases whose causal agents are transmitted by the water route. Typhoid fever statistics have provided some of the best evidence of the efficacy of treatment systems, but it should be kept in mind that other diseases, not so easily diagnosed, might also be kept under control at the same time. The so-called Mills-Reincke theorem held that, for every death from waterborne typhoid, there were several deaths from other diseases for which the causal agents were transmitted by water (Whipple, 1921). At present, the incidence of typhoid fever is so low in the United States that no useful information on the effectiveness of recent changes in water purification practices can be obtained from an examination of the statistics. During the years 1946-1970, there were 53 outbreaks of waterborne infectious disease due to typhoid, but there were 297 outbreaks due to other bacterial or viral agents (including 178 outbreaks of gastroenteritis of undetermined etiology (Craun and McCabe, 1973). Of the outbreaks seventy-one percent resulted from contamination of private water systems, but most of the illness (83%) was associated with community water systems. During the period 1946-1960 there were 70 outbreaks of waterborne disease in communities served by public utilities, (Weibel, et al., 1964) of which only 6 were typhoid fever. When data during this period for the number of outbreaks are examined, the incidence of typhoid is even lower--103 cases out of a total of 19,928 (for a percentage of 0.5%). Even considering that typhoid is more likely to be fatal than infectious hepatitis or gastroenteritis of unknown etiology, the Mills-Reincke theorem does seem to have considerable merit. Thus, the rationale that has been used in devising standards for microbiological contaminants (see quote above from the 1925 Standards) does not necessarily hold up to careful examination. The coliform standards may have ensured freedom from typhoid fever, but we do not have the same assuredness that they have guaranteed freedom from other infections. Even granted that most of the outbreaks reported have occurred because of breakdowns in the proper functioning of water systems, the results do show that intestinal infections other than typhoid are common, and because of their often ill-defined nature, may be improperly diagnosed. Finally, only "outbreaks" find their way into public health statistics, whereas sporadic, random cases of gastroenteritis generally go unreported. The epidemiological significance of the present microbiological standards warrants continuing investigation to bring about further refinements in meeting the goal of maximum public health protection.

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Executive Summary

The Safe Drinking Water Act of 1974 (PL93-523) required the Administrator of the Environmental Protection Agency to arrange for a study that would serve as a scientific basis for revising the primary drinking water regulations that were promulgated under the Act. The study was conducted by the Safe Drinking Water Committee of the National Research Council.

A thorough study of the scientific literature was undertaken in order to assess the implications for human health of the constituents of drinking water in the United States. Assessment of the health benefits and the economic or technological feasibility of achieving a given level of contaminant control is outside the scope of the study, although the beneficial effects of some constituents of drinking water were considered.

The risk to man of contaminants ingested in drinking water was evaluated on the basis of both epidemiological studies and studies of toxicity in laboratory animals. The theoretical and experimental bases for extrapolating estimations of risk to low levels of dose have been reviewed; some principles to guide the conduct of this and future studies are offered.

Five classes of contaminants were examined: Microorganisms, Particulate Matter, Inorganic Solutes, Organic Solutes and Radionuclides.

A great reduction in the incidence of gastroenteric diseases has resulted from the control of pathogenic microorganisms by the standard drinking water treatments (coagulation, sedimentation, filtration and disinfection) adopted early in this century. However, in 1975, more than 10,000 cases of waterborne enteric disease were reported, but in only about 10% of these cases were causal agents identified. There are reasons to believe that many cases go unreported. Improved detection and reporting systems are needed to determine more accurately the nationwide incidence and causes of these diseases. Chlorine is the standard disinfectant against which others are compared. While it is not ideal in every respect, much more research is required before any of the proposed substitutes can be recommended to replace it in water treatment. Questions concerning effectiveness of disinfection, toxicity of by-products, and residual in the distribution system must be answered for proposed substitutes, as well as for chlorine.

Finely divided solid particles are found suspended in many drinking water supplies, particularly in those not treated by coagulation and filtration. While certain particles may indirectly reduce the efficiency of disinfection treatments, and act as carriers of some other contaminants, only in the case of particles derived from asbestos minerals are there grounds for suspecting that direct effects on human health may result. Inhalation of asbestos dust for long periods of time has been shown to produce toxic effects, but evidence of the toxicity of ingested particles of asbestos minerals is not conclusive. Further research is necessary to resolve this problem.

Health effects associated with 22 inorganic solutes were reviewed. Most were judged to present little or no threat to human health either because of low concentration in drinking water, minimal potential toxicity, or both. Thirteen are essential nutrients. Their potential toxicity at high levels and nutritional role at lower levels complicate the issue, but none of them poses a threat to health at the concentrations normally found in drinking water. The inorganic contaminant with the greatest potential for toxicity is lead. The present standard may not provide an adequate margin of safety, especially for infants and young children. The data presented justify reexamination of the current standards for arsenic and selenium. The preponderance of evidence supports an inverse correlation between the incidence of cardiovascular disease and water hardness, but the underlying causal relationships are not clear.

On the basis of their relevance to the purpose of the study, 129 organic compounds (including 55 pesticides) were selected for detailed examination.

A list of the compounds in drinking water that are known or suspected carcinogens was prepared after a detailed analysis of the available data. Estimates of cancer risk to man from a lifetime exposure were made when sufficient data were available to permit a statistical extrapolation. These projections were made for 22 compounds judged to be either known or suspected human or animal carcinogens. Of these only vinyl chloride is confirmed to be a human carcinogen. The available data on mutagenicity and teratogenicity also were summarized.

Although the carcinogenic effects of the compounds were of primary concern, evidence of other effects was considered. An "Acceptable Daily Intake" (ADI) was calculated for 45 compounds that were judged to be potentially toxic but not carcinogenic. The ADI is an empirically derived value that reflects a particular combination of both knowledge and uncertainty about the relative safety of a chemical. It is the level at which exposure to a single chemical is not anticipated to produce an observable toxic response in man. The ADI does not represent a safe level in drinking water because it does not specify what fraction of the potential contaminant intake may come from water. Data were insufficient to calculate an ADI for 61 of the compounds that were considered.

The radiation associated with most water supplies is a small proportion of the normal backround to which all human beings are exposed. Consequently, it is difficult, if not impossible, to measure with certainty any adverse health effects that may be due to radionuclides in water. In a few water supplies, however, radium can reach concentrations that pose a higher risk of bone cancer for the people exposed.

Each chapter concludes with a summary and research recommendations. Subgroups have been identified that are more susceptible to the adverse effects of certain constituents than is the normal population-at-large.

Chapter I

INTRODUCTION

This chapter briefly outlines the general approach, principles and criteria used in the study. It is concerned with how the study was done, the basis for selecting the contaminants for consideration, and the factors entering into an evaluation of their health implications. This section is not a summary. Each chapter, except for the introduction, has its own summary.

The study was undertaken by the NAS-NRC to meet the needs expressed in the Safe Drinking Water Act (PL 93-523) which requires the Environmental Protection Agency to promulgate national drinking water standards and, for the first time, regulations for enforcing them. The Act also directs the administrator of the Environmental Protection Agency to arrange with the National Academy of Sciences, or other appropriate organization, to study the adverse effects on health attributable to contaminants in drinking water. Although the high quality of drinking water in the United States is recognized throughout the world, the law is an expression by the Congress of the concern of many citizens about maintaining the quality of public water supplies in this country.

The reader should not equate the size of this report or that of any of its chapters with the Committee's assessment of the magnitude of the challenge to public health that may be due to the presence of particular constituents in drinking water in the United States. Several factors have contributed to the length of this report: The Safe Drinking Water Act defined the scope of the study in encyclopedic terms and consequently the length of some of the chapters reflects the large number of topics and substances tht it was necessary to consider. Other chapters deal with subjects that are complex and about which there is uncertainty, conflicting opinions, and inconclusive or incomplete data. Summarization of relevant studies, assumptions, methodologies, health effects, and research recommendations for each group of constituents required detailed consideration from several points of view before a balanced judgement could be achieved. In some cases brevity had to be sacrificed to reach this objective within a reasonable time.

An evaluation of the public health implications of various contaminants has been the aim of this effort. The economic or technological feasibility of achieving a given level of contaminant control is outside the scope of the study. The health effects associated with some methods of disinfection have received attention. A comparative evaluation of the effectiveness and possible hazards associated with all the various methods of water disinfection has not been done.

Our interpretation of the intent of Congress is that the scientific community should give the best possible answers concerning the potential health effects of the various contaminants found in drinking water. For many of the known contaminants this cannot be done because the most current knowledge is too often inadequate and incomplete. The results reported here must therefore be considered as the first contribution to an effort that should be continued.

In considering specific contaminants, this report attempts to answer these questions:

1. What reason is there for concern about the material? What risks are there associated with its presence in water?
2. How does the material get into people?
3. What sources are there other than water?
4. What contaminants need to be controlled?
5. Are there special places or persons at higher-than-average risk?
6. Are there essential health requirements for this material? (This is dealt with in the discussion of trace minerals.)
7. In view of the data at hand, can one say that this is a material which causes temporary ill effects? Permanent ill effects? Reversible effects? What additional data are needed? (See Chapter II - "Chemical Contaminants: Safety and Risk Assessment".)
8. In view of these effects - and their reversibility (or lack of it), is it possible to set "no-adverse health effects" levels?
9. For materials with special health benefits, what level of these materials will maximize these benefits, while keeping the health risk associated with them at an acceptably low level?

Many of the materials considered here occur naturally, entering water from the rocks, the soil, and the air. Some of these are natural waste products of men or animals. Some are man-made--for other purposes--inadvertently finding their way into water. That a material is "natural" did not imply to us an automatic superiority or greater safety than for a material that was man-made.

Volume of water consumed

In this study, a quantity of two liters per day has been used as the average amount of water consumed per person. This is the amount used by EPA to calculate the current interim standards. The volume of water consumed by a person is a function of the temperature, humidity, physical activity, and other factors which vary widely throughout the country. The average per capita water (liquid) consumption per day as calculated from a survey of nine different literature sources was 1.63 liters. (NAS, 1974; McNall and Schlegal, 1968; Wolf, 1958; Guyton, 1968; Evans, 1941; Bourne and Kidder, 1953; Walker *et al.*, 1957; Randall, 1973; Pike and Brown, 1975.) Persons consuming larger volumes than two liters per day still are afforded adequate protection because the margin of safety estimated for the contaminants is sufficient to offset excess water consumption. Water consumption of two liters per day has therefore been taken as applicable to the majority of water consumers. Perhaps consideration should be given to establishing some standards on a regional or occupational basis to account for extremes of water consumption.

Risk and Safety

The hazards of ingesting chemical pollutants in drinking water have been assessed in two general ways: with laboratory toxicity studies and epidemiologic studies. The aim of studies of both types is to provide information about the risk to man. Risk constitutes only half of the equation; the other half is--benefit to the exposed population. It is not possible to guarantee a risk-free society. The scientific methods and criteria we have used for evaluating long-term effects and risks in man are described in the chapter on Chemical Contaminants: Safety and Risk Assessment and in the chapters concerning each group of contaminants. Most of the experimental results on which the current knowledge of toxicity rests are based on observed effects on man and animals of doses and dose rates that are much larger than those that correspond to the usual concentrations of harmful materials in drinking water. There is, consequently, great uncertainty in estimating the magnitude of the risk to health that ingestion of contaminants in water may produce. An additional problem is to take into account the combined effects of two or more contaminants.

The theoretical and experimental bases for extrapolating estimations of risk to low levels of dose have been reviewed, and some principles proposed to guide the conduct of this and similar studies.

Microbiological contaminants

Outbreaks of waterborne disease are reported to the National Center for Disease Control (CDC) by state health departments. In addition, EPA obtains information about additional outbreaks from state water supply agencies. Both CDC and EPA are aware that data on waterborne outbreaks have limitations and must be interpreted with caution. The data collected represent only a small part of a larger public health problem. The number and kind of reported outbreaks and of some etiologies may depend upon the interest or capabilities of a particular state health department or individual. They do not reflect the actual number of outbreaks, cases, or etiologies of disease associated with drinking water.

Many small outbreaks are not reported to state health departments. There is no law or regulation requiring state authorities to report all gastroenteritis cases to CDC. In 1975, CDC reported 24 waterborne disease outbreaks involving 10,879 cases. No etiologic agent was found for the two largest outbreaks (Sewickley, Pa., 5000 cases and Sellersburg, Ind., 1800 cases). In fact, no etiologic agent was identified in 17 of the 24 outbreaks. These 17 outbreaks accounted for 9,760 cases, or 89% of the total reported in 1975.

Conclusions in the microbiology chapter, based on epidemiological data, are subject to the limitations of the reporting system and to our limited ability to identify the etiologic agent in outbreaks known to be associated with drinking water.

The microbiological contaminants selected for consideration in this report are those for which there is epidemiological or clinical evidence of transmission by drinking water. These include a variety of bacteria, viruses, and protozoa. Consideration is given to how these contaminants are detected in drinking water and an evaluation of minimal permissible levels is made. Because current drinking water standards place major emphasis on detection of microbiological contaminants, there is considerable attention devoted to the validity and health significance of microbiological standards.

Inorganic solutes

The Interim Primary Drinking Water Regulations list maximum allowable concentrations for six metallic elements - barium, cadmium, chromium, lead, mercury, and silver. Ten additional metals were reviewed in this study - beryllium, cobalt, copper, magnesium, manganese, molybdenum, nickel, tin, vanadium, and zinc. Sodium, which is also a metal, was considered separately, because the problems it poses are quite distinct from those associated with the other metallic substances. In addition, the effects on health of several other inorganic constituents of drinking water were also studied. These include arsenic, selenium, fluoride, nitrate and sulfate. The relationship between water hardness and health also received attention.

The sources of inorganic ions in ground water, surface water, water-treatment chemicals, and from the storage and distribution system are considered along with the health effects resulting from the total intake from food, air and water.

Organic solutes

Of the 298 volatile organic compounds so far identified in drinking water, 74 were selected for detailed study along with 55 pesticides. A compound was selected for consideration if any of the following criteria applied:

1. Experimental evidence of toxicity in man or animals, including carcinogenicity, mutagenicity, teratogenicity.
2. Identified in drinking water at relatively high concentration.
3. Molecular structure closely related to that of another compound of known toxicity.
4. Pesticide in heavy use; potential contaminant of drinking water supplies.
5. Listed in the Safe Drinking Water Act or National Interim Primary Drinking Water Regulations.

The evaluation of toxicity was based on a critical review of the scientific literature. The available data were of variable quality and quantity and in some instances, inadequate for proper assessment of toxicity. In those cases where sufficient data were available, professional judgement was used to determine which compounds are carcinogenic, mutagenic, teratogenic and noncarcinogenic.

The limitations that are inherent in the extrapolation of high-dose animal bioassay data to low-dose human exposure and the difficulty of making predictions for species which may have different metabolic rates and pathways for handling carcinogens and/or different target-organ responses are well known. Such risk assessment and extrapolation procedures are further compromised by the limited information which is available concerning the mechanisms by which these agents act (such as initiators, promoters, and modifiers) and the almost total lack of data regarding the potential synergistic and antagonistic interactions of these agents with each other and with other environmental agents. The risk of ingesting known or suspected carcinogens was estimated by the methods described in Chapter II. These methods are based on an assumption that there is no threshold in the dose-response relationship. The risk-estimate approach may provide unique advantages for other areas of toxicologic evaluation.

The more traditional approach of combining the maximum no-observed-adverse-effect level with an uncertainty (safety) factor to calculate an acceptable daily intake (ADI) was used for agents which were not considered to be known or suspect carcinogens and for which there was adequate toxicity data from prolonged ingestion studies in man and/or animals. Several alternative terms, other than ADI, were considered, but it was concluded that the introduction of new terms might lead to confusion, and that the use of a widely-recognized and generally-acceptable term would be preferable for this report. Although the ADI has been used previously as an internationally-established standard for the toxicologic evaluation of food additives and contaminants, the concept is applicable to other ingestion-exposure situations. The ADI represents an empirically-derived value which reflects a particular combination of knowledge and uncertainty concerning the relative safety of a chemical. The uncertainty factors used to calculate ADI values in this report represent the level of confidence which can be justified on the basis of the animal and human toxicity data. ADI values were not calculated for agents where the data was considered to be inadequate.

Since the calculation of the ADI values is based on the total amount of a chemical ingested, the ADI values calculated in this report do not represent the safe level for drinking water.

Little or no data are available on the toxicity of many organic compounds identified in drinking water. There is a need to determine which of these compounds should be subjected to extensive toxicity testing. Some of the criteria used for developing the order in which compounds should be tested are:

1. The relative concentrations of the compounds and the number of people likely to be exposed;
2. The number of supplies in which they occur;
3. Positive responses in _in vitro_ mutagen screening systems;
4. Positive responses in _in vitro_ pre-screening systems for potential carcinogens. (Mammalian cell transformations);
5. Similarity of the chemical structure of the test compound with that of other compounds having defined toxic properties (i.e., structure-activity relationships); and
6. Relationships of dose from water to total body burden.

Particulate contaminants

Finely-divided solid particles of mineral and organic composition are commonly found suspended in some drinking water, particularly those supplies which do not practice coagulation and filtration. To discover whether or not the long-term ingestion of these materials in water is likely to produce adverse effects on human health, their occurrence, composition, and properties were reviewed.

This review indicated that many kinds of particulate matter may indirectly, through adsorption, facilitate the transport of toxic substances and pathogenic organisms, and affect the efficiency of disinfection. Particles of organic composition also may indirectly give rise to chlorinated compounds by reaction with chlorine in water treatment.

Only in the case of particles derived from asbestos minerals, however, are there grounds for suspecting that direct effects on human health could be involved. Fibrous particles of asbestos minerals are known to be associated with increased incidence of cancer, including gastrointestinal cancer, among workers who inhale asbestos-laden air. Inhalation experiments with animls using asbestos mineral fibers have also demonstrated a carcinogenic effect. The particulate matter in drinking water often includes similar particles.

Although epidemiological studies have not indicated an increase with time in cancer death rates that can be ascribed to fibrous contamination of the drinking water, these negative findings do not exclude the possibility that such an increase might be detected in the future because many cancers have long induction periods.

For these and other reasons, detailed elsewhere, it is believed to be important that research on the analysis of fibrous mineral particles in water and on the toxicity of these materials when ingested, should be strongly pursued.

Radioactive contaminants

Because the presence of ionizing radiation is one of the standard features of the earth's surface, the adverse effects on health that may be ascribed to radioactive contaminants of drinking water were assessed in relation to the average background radiation dose, from all sources of 100 mrem per year.

Previous estimations of the biological effects of the background radiation on human health were reviewed in the light of more recent scientific knowledge and used to calculate the magnitude of three kinds of adverse health effects that radiation can produce; namely, developmental and teratogenic effects on the fetus; genetic disease; and somatic (principally carcinogenic) effects.

When these estimates are related to the concentrations of radionuclides that are commonly found in drinking water, it is seen that consumption of two liters of water per day contributes such a small fraction to the total radiation background that the incidence of developmental, teratogenic, and genetic disorders is not increased enough for the change to be detectable.

Where somatic effects are concerned, it is estimated that the radionuclides in drinking water typically account for less than 1% of the incidence of cancers that may be attributed to the natural background of radiation. Only certain bone-seeking radionuclides (chiefly radium), in a few regions, reach concentrations in drinking water that are high enough to cause a significant increase in the incidence of bone cancer.

Susceptible subgroups and other considerations

Groups which are more susceptible than the normal population are considered in the chapters on various classes of contaminants.

This report is concerned only with water used for drinking and not for other purposes. Although the use of water in health care facilities presents problems that concern all classes of contaminants, the health hazards associated with such diverse uses of water as in: humidifiers; kidney dialysis units; laundries; heat-transfer in heating and cooling equipment; or many special uses that require further treatment of tap water, have not been considered. References and summaries of the scientific literature in this field have been published (DeRoos et al., 1974).

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Chapter II

CHEMICAL CONTAMINANTS: SAFETY AND RISK ASSESSMENT

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Chapter II

CHEMICAL CONTAMINANTS: SAFETY AND RISK ASSESSMENT

The hazards of ingesting pollutants in drinking water can be assessed in two general ways: with studies of toxicity in the laboratory, and with epidemiological studies. Studies in the laboratory may employ a variety of experimental systems, ranging from chemical effects of pollutants on DNA, through exposure of bacterial or mammalian cells in culture, to lifetime feeding studies on experimental animals. They are prospective studies, in which relatively small numbers of cells or animals are exposed to characterized pollutants at known concentrations. Epidemiological studies deal with human populations; their design is constrained by external circumstances, and they involve large numbers of people whose exposure to the pollutant in question is commonly uncertain and confounded by exposure to other pollutants.

The aim of studies of both types is to allow the risk to man to be estimated. The first can give precise information on relatively high risks related to individual pollutants in this or that animal species--to which human exposure and risk may be compared. The second can provide less precise information on the human risk related to one pollutant (isolated, it is hoped, from other pollutants).

Toxicity data obtained from laboratory animals will generally have to be relied on for estimating human risk, if we are to control human exposure to carcinogens. Epidemiological studies have discovered causes of disease and can buttress, supplement, or contradict laboratory data. Imaginative comparisons between laboratory and epidemiological data are of the utmost importance, particularly in the area of metabolic pathways and fate of chemicals found to be animal carcinogens.

Efforts to develop rapid assays for mutagenesis and carcinogenesis have recently been greatly expanded. Methods that show promise include tests for mutagenicity that make use of bacterial, cell transformation, and organ culture systems. There appears to be high positive correlation between mutagenicity, as determined by some of these methods, and carcinogenicity in agents already studied (McCann *et al.*, 1975; McCann and Ames, 1976; Ames, 1976). The utility of these rapid methods will depend on experimental demonstration that their results are well correlated with those obtained from conventional long-term studies of carcinogenicity with well-designed animal systems. High priority should be given this research because it offers a reasonable probability of success in a relatively short time and at lower cost than long-term testing, and there is an urgent need for a primary screen for selecting compounds for long-term assay (DHEW, 1977).

The committee is fully conscious of these modern methods for determining genetic and pharmacological phenomena. Their use when appropriate, and their further development, is strongly encouraged.

Pollutants in water have many different effects. At one extreme, they can impart a disagreeable taste or odor. This is quickly perceived by the community, and the process of characterisation and identification of the offending pollutant is generally prompt and fairly straightforward. At the other extreme, the effects on human health of a carcinogen present in drinking water will probably go undetected, particularly if it produces only a modest increase in the incidence of a common cancer.

The major toxicological and epidemiological efforts should therefore be directed to characterising and identifying pollutants whose biological effects include chronic, irreversible, and progressive diseases, such as cancer. It is necessary to develop risk estimates for large human populations of varied susceptibilities that are exposed to small concentrations of such toxic pollutants, including carcinogens.

The development of safety factors for pollutants whose toxic effects are reversible and nonprogressive involves empirical calculations based on past history of use and concentrations that appeared safe for the public. These safety factors are usually applied to the highest dose or concentration at which no adverse effect was observed. The chosen dose or concentration is divided by a "safety factor" that varies over a wide range, depending on the adequacy of the data.

Whether or not the "safety factor" approach can be used with pollutants that cause chronic, irreversible, and progressive disease in laboratory animals is controversial. Those who argue for the use of safety factors, and the threshold doses that these depend on, find it inconceivable that very small concentrations can cause a cancer to develop, claiming that body defenses can surely protect at doses smaller than the threshold value. Those who argue that safety factors are inadequate and that almost no thresholds can be determined, or theoretically developed, suggest that even one or a very few molecular events have some probability of initiating a malignant or neoplastic transformation in a cell, and that this can lead to a lethal cancer. Although one malignant cell can lead to death by cancer, many liver or kidney cells can be killed or damaged (but not malignantly transformed), without causing any detectable disease.

Furthermore, man is never exposed to one carcinogen at a time, but is exposed to low concentrations of many at the same time.

Accordingly, we have adopted a 'nonthreshold' approach for estimating risks from pollutants that have been shown to be carcinogenic in laboratory animals.

Demonstration that a pollutant is carcinogenic, and application of nonthreshold risk estimates to it, do not imply that its use must be prohibited. Such a proscription might itself give rise to even greater risks to health, or other disadvantages. In some cases, a net risk must be estimated, and society must attempt to use the pollutant in such a way as to minimize risk and maximize benefit. Nowhere is this better illustrated than in the use of chlorination to disinfect water. Chlorination controls pathogenic organisms, but introduces chloroform, which is carcinogenic in animal-test systems. Methods must be devised to minimize concentrations of chloroform and chlorinated hydrocarbons, from whatever source, in drinking water. But before alternative methods for control of pathogenic organisms are instituted, toxicological studies must show that they are as effective as, and pose no greater risk than chlorination. We perceive that society is willing to accept some risks to health if the attendant benefits are demonstrably greater.

Drinking water contains low concentrations of many chemicals, some of which, if ingested for a long time, could have delayed toxic effects. The insidious effect of chronic exposure to low doses of toxic agents is difficult to recognize, because often there are few early warning signs and, when signs are ultimately observed, the effects may have become irreversible. Subchronic toxicology studies may not offer reliable means for assessment of long-term toxic effects in animals, let alone extrapolation to chronic effects in man; hence, different considerations have to be applied in assessing risk. The methods and principles of acute toxicology do not offer any easy, straightforward methods for extrapolation of such experimental data to calculate risks for large human populations.

Two important questions must be answered: What assay procedures are required for a valid assessment of chronic toxicity of chemicals in experimental animals? How can the data from such procedures be extrapolated to estimate risks in humans? In dealing with these questions, we use the specific risk of cancer as our major example, although other toxicities are considered. This report seeks to summarize the state of the art in extrapolating to man the results of experiments on animals, chiefly in relation to chemical carcinogenesis.

EFFECTS ON HEALTH

The purpose of drinking-water standards is to ensure protection from acute poisoning and from long-term, or "chronic," effects. In recent years, numerous short-duration, presumptive tests *in vitro* have been developed that may help to predict carcinogenesis. Nonetheless studies of chronic toxicity continue to be required for safety evaluations. These are necessary because there is no general way to predict carcinogenic effects on the basis of the observed short-term effects of chemical-biological interactions.
(The significance to health of the finding that a pollutant is mutagenic in the new test systems is unknown. (See Drake, 1975; DHEW, 1977) But, because evidence of the correlation between mutagenicity and carcinogenicity continues to accumulate, we suggest that a conservative safety factor be provisionally applied to the mutagenicity data and that, if new information (such as the results of a reliable carcinogenicity study) is lacking 4 years from the time a mutagenicity study is completed, nonthreshold methods be used to establish risk.)

Chronic exposures and chronic effects are different (Casarett, 1975). The former means frequent ingestion over a long period of time. The latter implies injury that persists, either because the injury is irreversible or progressive or because the exposure is prolonged and the rate of new injury exceeds the rate of repair. Chronic exposure in animals is generally considered to be at least half the lifespan. In man, it can be much less.

Injury from chronic exposure may occur in at least three ways: by accumulation of the chemical to a critical concentration at sites of action sufficient to induce detectable injury; by accumulation of injury until physiological reserves can no longer compensate (i.e., repair is never complete); or after a long latent period beginning with an exposure that has an unrecognized biological effect, precipitating the eventual appearance of injury. In the first case, knowledge of the kinetics of chemical absorption, metabolism, and excretion obtained in short-term studies may allow computation of the amount of the toxic chemical that will accumulate in long-term use. Such studies will improve the usefulness of long-term, low-dose, chronic-exposure studies. To predict chronic accumulation, or latent development, of injury from the results of short-term tests requires knowledge of the kinetics of injury and repair. There are few, if any, substances for which such understanding is at hand.

Reversibility of Chemical Injury

Reversible effects disappear after exposure ends. The time required for return to normal should be a small fraction of the remaining lifetime of an organism. During the period of return to normal, the organism must be at no greater risk (th[illegible] one that was never exposed) of further or other damage from oth[illegible] sources. For some effects, reversibility may be qualified by t[illegible] normal lifetime of a specific cell or macromolecule that serves as the endpoint for the effect. A nonreversible effect is one in which the damage does not regress completely, or may progress after exposure ceases.

Some effects of toxic chemicals are unmistakably irreversible. These include terata, malignant tumors, mutations in offspring of exposed animals, and some neurological changes. These are gross manifestations of specific chemical interactions with cells, and it is possible, or probable, that there are early reversible effects either in the cellular process first affected, or at intervening stages. Prediction of adverse effects from short-term studies is possible if the critical dose and the rate-limiting factors that determine reversibility are known. Without this knowledge, evaluation of toxicity will generally deal more with the possibility of irreversible effects than with speculatively reversible effects.

Net reversibility varies from one tissue, species, strain, or individual to another. It is generally impossible to measure the specific processes involved in injury and repair in the standard toxicity-evaluation study. However, measurements of reversibility in short-term studies should provide useful information that might allow extrapolation to the longer term.

The predictive value of such tests does not necessarily depend on the persistence of the chemical in the test organism. If the chemical produces a reversible effect and then is rapidly detoxified or excreted, it may be possible to compute the doses or schedules of exposure that would not produce cumulative and ultimately nonreversible or irreversible effects (see definitions below). But other factors might be overriding. For example, rapid reversibility after a single dose might not indicate the rate of reversal after repeated doses, if the first dose, in addition to the measured effect, altered the repair process or processes responsible for detoxification (Murphy, 1967). To evaluate repeated-dose effects, subchronic- and chronic-exposure studies should include groups of animals that are removed from exposure at selected intervals during the experiment. The rate of reversal of effects in these animals can be measured at intervals, or at some critical time after exposure ceases.

If the chemical persists in the organism, quantification of rates of reversal is more complicated. Data are needed on absorption and disposition to correspond with data on rates and reversal of effects.

Perspectives and Perceptions of Effects

Whether an effect is reversible, nonreversible, or irreversible, might be shown by experiments (or epidemiological studies) that include observations during exposure-free "recovery" periods similar to those made at the end of the exposure period. Nonreversible and irreversible injuries are of greater concern than reversible injuries in evaluating human health effects. However, frequently recurring reversible injury may lead to greater morbidity than a nonreversible or irreversible injury that appears only late in life. Characterization of an effect as reversible implies that there is a dose below which health will not be compromised. This assumes, of course, that any subliminal cellular injury that is responsible for the manifest effect is also reversible. Full understanding of thresholds, margins of safety or safety factors, and extrapolations of estimated risk, requires understanding the underlying cellular mechanisms of injury.

An alternative to the safety-factor approach for reversible toxicity may be considered. The nonthreshold approach is attractive partly because of the idea that one transformed cell could lead to fatal neoplastic disease. What number of damaged (but not transformed) or killed kidney, liver, or lung cells, is compatible with a healthy life? If these numbers, or fractions of total organs, could be estimated for a number of species, including man, and if experimental dose-response curves for fractional damage to all vital organs could be obtained, the numbers of damaged cells that are compatible with health could be estimated. This might constitute an initial approach to the development of rational risk estimates for toxic effects other than cancer. Clearly, a major research effort is needed.

Where it was once common to refer to "no-effect doses" of chemicals and "safe" doses, it is now more appropriate to speak of "no-observed-adverse-effect" doses and "acceptable risk", when describing permissible use or exposure to chemicals. This change has been accompanied by an increasing concern for the health of the most susceptible individuals in the population, besides that of the average individual. Many scientists now distinguish between injuries produced by chemicals for which there is likely to be a threshold dose, and effects (e.g., carcinogenesis and mutagenesis) for which there is likely to be either no threshold dose, or no way presently known to estimate one for large, heterogeneous populations. [A report of another committee of the National Academy of Sciences expresses some doubt about the validity of the threshold concept for any type of biological effect (NAS, 1975); see also Drake, *et al.*, 1975, and Hoel, *et al.*, 1975.] From another point of view, Weil (1972), considering statistics and judgment in safety evaluation, wrote: "No matter what the biological effect, at some concentrations under some sets of conditions, a dose level must exist below which no biological damage will occur during the life-span of the great majority of men. No matter how small the dose, however, one, or a few, of millions of subjects may exhibit the critical response."

It is more prudent to treat some kinds of toxic effects that may be self-propagating or strictly cumulative or both, as if there were no threshold, and to estimate the upper limits of risk for any given exposure. Included among these are effects that result from an initial, chemically-induced alteration in cellular genetics that is transmitted by cell propagation. Carcinogenesis and mutagenesis are examples in which a single cell transformation has the theoretical potential for irreversibility, which might involve self-propagation even in the absence of further exposure.

Other injuries may become self-propagating--e.g., advanced stages of cirrhosis--but they are usually preceded by detectable injury that is reversible. The initial effects should have a dose-response threshold, inasmuch as the cellular injury that precedes them can be detected while the injury is still reversible.

Some forms of injury may be strictly cumulative, because the cells in which they occur are not repaired or replaced. (For example, destruction of enough neurons leads to a decrease in central nervous system function.)

Congenital malformations appear to be irreversible. In this case, injury occurs from exposure during only a brief period. In addition it is probable that a threshold dose could be estimated from adequate experimental or epidemiologic data.

Current knowledge of the proper principles for extrapolating toxicological data from high dose to low dose, and from one species to another is inadequate. Nonetheless, standards for drinking water must be developed. Whenever possible, a maximal no-observed-adverse-effect dosage should be identified. Three major categories of effects should be considered, and different ways of arriving at standards can be proposed for each.

I. Irreversible (Self-Propagating) Effects

(These are likely to become life-threatening even after exposure has ceased)

1. Genetically self-propagating effects, e.g., somatic or germ-cell mutation that culminates in a malignant neoplasm or is transmitted to later generations: Assume no threshold, assume a linear dose-response at low doses, and estimate risk. Set standard at something other than zero only if exposure cannot be eliminated by reasonable means, or if the material has no safer

substitute, and if it has great utility or social value. An acceptable degree of risk arrived at by a case-by-case consideration involving numerous scientific, technological, economic, and societal issues and values should determine the permitted dose.

II. Nonreversible Effects

1. Effects that are sequels to probably detectable, reversible injury, but that may become self propagating (such as cirrhosis): If a threshold can be demonstrated, use it as an upper limit, with application of an appropriate safety factor. If not, proceed as in 1.--"Irreversible Effects."

2. Death of irreplacable cells, that are cumulative with continued exposure, e.g., central nervous system disease, as in exposure to methyl mercury: If a threshold can be demonstrated, use it as an upper limit, with application of an appropriate safety factor. If not, proceed as in 1.--"Irreversible Effects."

III. Reversible Effects

1. Life-threatening or major morbidity, e.g., inhibition of a vital enzyme system: If a threshold can be demonstrated, use it as an upper limit. If not, proceed as in 1.--"Irreversible Effects."

2. Minor morbidity, e.g., sensory irritation without histological change: Determine the range of sensitivities and choose an upper limit low enough to minimize occurrence in the population.

3. No detectable functional or sensory decrement, but possibly predictive precursor of more serious effect, e.g., plasma cholinesterase inhibition, or small increase in liver enzymes in plasma: Proceed as in 2.--"Reversible Effects."

IRREVERSIBLE TOXICITY

Many factors make the assessment of long-term health risks to human populations difficult--for example:

1. The sensitivity of the test systems used to detect carcinogenic effects depends on the number of animals used in each test and on the duration of their survival.
2. Any series of experiments will yield false-positive and false-negative results.
3. Detection of neoplastic changes in treated animals requires extensive gross and microscopic examination of many tissues by trained people.
4. Time, resources, and money required to conduct an adequate test are all substantial.

Controversies have arisen because of the above problems, and because of inadequate testing for long-term effects. False-positive results can cause unnecessary public concern and the removal of useful materials from the market, and false-negative results can endanger the health of large groups of people. Long-term effects are particularly difficult to detect and treat because they are discovered only after many years, by which time they are often irreversible.

The main question to be answered is: "Within the limitations of preset-day capabilities, what are the minimal requirements for an adequate test (on experimental animals) of the long-term effects of potentially toxic agents, and how can these results be used to estimate possible risk to the human population?"

Summary of Principles for Extrapolating Animal Toxicity To Humans

Despite wide gaps in our knowledge of the metabolism and ultimate fate of chemicals in man, properly conducted experiments will yield results that can improve our estimates of the risk to human populations from long-term exposures.

Many mechanisms for chemical carcinogenesis have been postulated. If the mechanism involves somatic mutation or alteration, there is no threshold dose for long-term exposure; if the mechanism is unknown, it is prudent to assume that DNA damage is involved. The idea that there is a "safe" dose of such chemicals may be conceptually valid, but "safety" can not be established by any experimental method now available. Every dose should be regarded as carrying some risk. A "most probable risk" can be estimated by appropriate statistical treatment of the results of experiments on animals, and once the benefits of use of a chemical have been defined and estimated, it is possible to weigh the health risks against the health benefits. The balance between them should then be the overriding consideration in regulating the amounts of such substances in the environment.

The method used in classical toxicology for determining safe doses for short-term exposure of humans to drugs is to estimate a maximum exposure that is tolerated without adverse effects in a group of animals, and to apply a safety factor. This procedure is valid only for estimating the risk of reversible toxic effects. "No-observed-adverse-effect dose" is a better term, because it makes clear that the exposure can often be a function of the size of the experiment; the larger the experiment, the lower this dose can be.

Studies in laboratory animals must be used to predict the safety of environmental chemicals. Human epidemiological studies can not be used to predict nor assure safety, for several reasons:

1. Epidemiology cannot tell what effects a material will have until after humans have been exposed. One must not conduct what might be hazardous experiments on man.
2. If exposure has been ubiquitous, it may be impossible to assess the effects of a material, because there is no unexposed control group. Sometimes, historical data can be used, i.e., by asking what the state of illness was before the new material was introduced. For chronic effects, with variable latent periods and overlapping of introduction and removal of materials, historical data are not usually satisfactory.
3. It is usually difficult to determine doses in human exposures.
4. Usually, it is hard to identify small changes in common effects, which may nonetheless be important if the population is large.
5. Interactions in a "nature-designed" experiment usually can not be controlled.

With the possible exception of arsenic and benzene, the known human carcinogens are carcinogenic in some laboratory species. Therefore, animal studies of carcinogenesis in laboratory animals are useful for predicting effects in man.

Thus, for ethical and practical reasons, data derived by using animals for toxicity testing are essential for protecting the public from harmful effects of new chemicals in the environment, and probably also are necessary for evaluating the potential harm of "old" chemicals. By the same token, epidemiological surveillance studies are necessary in order to to detect the errors that will surely arise from the animal studies alone. Thus, epidemiological studies are both a last line of defense and a mechanism for verifying and adjusting the animal studies.

The General Problem of Extrapolation

The knowledge and insight that provide a basis for more successful extrapolation are rapidly increasing. The value of tests on laboratory animals is most easily estimated when the chemical agents tested are ultimately administered to or confront man in a manner similar to the animal exposure, such as in the drug-development process. The sequence of animal tests of a new chemical agent, after toxicological studies, continues with studies conducted in order to determine: the mechanism through which the laboratory animals respond to the agent; the nature of its metabolism in tissues and organs; and the rates and routes of elimination of the agent and its derivatives. Thus, damage observed in an organ of an animal provides clues that lead to an understanding of the metabolism and organ involvement of the substance in humans. Similarities and differences between humans and animals can be noted, and the validity of the laboratory-animal test systems can be better estimated. This approach is most useful for observing early effects that occur soon after the substance is administered. The use of such data for assessing long-term effects in humans has many difficulties.

When a mouse or man is exposed to a chemical, a number of events can occur that can greatly influence the final reaction, which may appear as the observed toxic effect. These events include: absorption; distribution and storage; metabolism, excretion and reabsorption; arrival at the site of action; reaction with the biological receptor; and interaction with other constituents of the environment. These events can be compared among various animal species and among strains and individuals. Anatomical, biochemical, physiological, pharmacological, and pathological differences and similarities can and have been identified, and there have been efforts to characterize systematically the differences and similarities between species for some compounds and classes of compounds. These are appropriate subjects for research.

Chemicals to which man is exposed can be divided into two classes: those deliberately administered for therapeutic, diagnostic, or nutritive purposes, which thereby directly contribute to health; and those with uses that do not directly benefit human health, but reach the population through a variety of routes.

To some extent, the acute toxicity of the first class can be observed directly in man. If the chemicals are already in use, the laboratory-animal toxicity tests provide a type of pre-screening or early warning system. However, chemicals of the second class are generally not tested in man, and their potential hazard can be estimated only through tests on animals epidemiological studies, or observation.

Extrapolation of data from laboratory animals to man is difficult to treat in a systematic and comprehensive fashion. Although vast amounts of data have been accumulated on the toxicity of compounds in animals and the effects of drugs and other chemicals on human health, experiments that generate the data tend to be so diverse that comparison is usually impossible. Good quantitative data on the toxicity of compounds in man are rare. Most commonly, there is neither knowledge of the amount of a chemical to which man was exposed nor of the incidence and severity of toxic effects.

Toxicologists rely heavily on "experience" for successful extrapolation of toxicity data to man. Unfortunately, experience is interpreted differently by different people and thus is a variable guide. For some of the most serious toxic effects, it can also be very misleading. For example, some cancers may take decades to develop in man after exposure to a carcinogen, so it is difficult for investigators to develop experience that will permit them to link exposure to ultimate effect. Tests for mutagenic effects in the human population are inadequate today, and it is possible that such effects from chemicals are already appearing. However, who has had enough "experience" to infer that man has changed because of some specific chemical in, say, the last 50 years? Statistical observation of small changes is usually difficult. For example, if 20% of all pregnant women used a chemical that caused stillbirths in 5% of the women who used it, the resulting increase in stillbirths would be 1% (0.20 x 0.05 = 0.01), and it is likely that it would not even be noticed. If 5% of all pregnant women used a chemical that caused a delayed effect in 20% of their offspring, this also would probably escape notice. Even if the chemical caused a 10% increase in a most common form of cancer in the offspring of the 5% of women (e.g., cancer of the colon, which would mean an increase of 200 deaths per year), the effect would very likely go undetected.

Specific Problems in Extrapolation

Experiments on laboratory animals are generally performed under highly standardized conditions, with controlled diet, temperature, humidity, and light-dark cycles, and usually with genetically homogeneous animals, such as inbred mice, rats, or beagles. Thus, one obtains reasonably precise and reproducible information on the toxicity of a substance under specified conditions, in animals from specific genetic pools. However, large and diverse segments of the human population may be exposed to the compound. Humans are not genetically homogeneous; they live under various environmental conditions; they eat a great variety of foods; and they are exposed to large and increasing numbers of substances. We would like to protect the whole population--its most sensitive members as well as the average.

Therefore, environmental and genetic variability must be considered in the process of extrapolation.

Man is generally exposed to toxic pollutants in the water supply through his gastrointestinal tract, and laboratory animals should be exposed in the same way. However, some problems inherent in the use of animals must be kept in mind when animal studies are used to predict the carcinogenic potential of a substance for man.

The Size Of Animals. Size tends to determine the rate of distribution of substances in the body; in large animals, they are distributed more slowly and tend to persist longer. In general, large animals metabolize compounds more slowly than do small animals. Large mammals have many more susceptible cells; thus, if the carcinogenic event is rare, it would be more likely to occur in a large mammal than in a small one (a mouse experiment using 1,600 - 2,000 animals represents about the same number of susceptible cells as are contained in one man). Obviously, however, a mouse is not the equivalent of a very small man; nor is a man the equivalent of a very large mouse. In the mouse, the ratio of cardiac output per minute to blood volume is 1:1. In man, the ratio is 1:20. Thus, blood circulates approximately 20 times as rapidly in a mouse as in a man. (This difference is not a peculiar species difference, but typifies the differences between very small and relatively large animals.) The implication is that the time a substance spends in the plasma (excluding metabolism) is longer in a larger mammal than in a smaller one. Thus, for the same milligram-per-kilogram dose, human tissues are exposed to a substance for a much longer time than mouse tissues. This is consistent with data obtained in studies of anti-cancer drugs, which showed that--on a milligram-per-kilogram basis--a mouse required 12 times as much drug to respond as did man, a rat 6 times as much, and a dog and a monkey 2-3 times as much. When the data were expressed on a milligram-per-square-meter basis, the differences between species were sharply reduced. In addition, the rate of cell division is greater in small animals; for example, the cycle time of mouse gut or marrow cells is about half that of man. The life span of a man is 35 times that of a mouse. Thus, there are many more cell divisions in man, and therefore a greater opportunity for neoplastic change.

These observations suggest that small mammals that are routinely used for toxicity testing are often more resistant to toxic compounds than man. This implies that small animal systems are likely to produce many false negative results, and has important implications for establishing safety factors or using "conservative" techniques for extrapolation.

Populations. The number of exposed animals is important. Typically, about 10^2-10^3 experimental animals are tested, whereas the population of humans to be protected may be 10^6-10^8.

The human population is genetically heterogeneous, whereas test populations of animals, as a rule, are relatively inbred. Genetic traits can affect many aspects of susceptibility to pollutants.

Selectivity influences the test-animal population in that only healthy, vigorous animals are started on test, whereas the exposed human population may contain sub-populations that are ill and weak.

Environmental Differences. Nutritional differences and the physical environment (e.g. heat, light, ionizing radiation,) can affect response to pollutants. The chemical environment--from drugs to air pollutants--with its overwhelming number and types of substances, provides the possibility for synergistic toxicity. Synergistic effects have occurred with therapeutic drugs, are well known in experimental carcinogenesis, and are an ever-present danger.

Absorption. Rates of absorption of chemicals through the gastrointestinal tract are generally comparable in laboratory animals and man. The barriers within the gastrointestinal tract and the cell membranes, which prevent the free passage of organic compounds, are quite similar among mammalian species. These barriers are either the cells themselves or the intercellular junctions, which are "closed" or "tight" and which, in general, force substances to move through cells, rather than between them. It should be recognized, however, that there are differences between one animal and another and between man and animal. The ratio of surface area to volume in the gastrointestinal tract, and the transit time through it, often differ among and within species. These factors affect absorption. The pH differs in various parts of the gastrointestinal tract in different species; this can affect the absorption of some partially ionized compounds. The possibility that intact proteins can be absorbed in neonates of some species suggests that absorption in the neonate can be nonspecific.

The presence of bacteria in the gastrointestinal tract can affect absorption indirectly. Bacteria may convert the original substance into one that is more or less absorbable and thus alter the apparent toxicity of a chemical, or they may convert a nontoxic substance into a toxic one. For example, reduction of nitrate to nitrite permits the formation of highly carcinogenic nitrosamines by reaction with secondary amines from the diet. The bacterial populations in the gastrointestinal tract vary between species and within species, and in the same individual from time to time, often changing with changes in diet.

Distribution And Storage. Once a compound is absorbed, it is distributed throughout the body, largely by the circulatory system. This gives the opportunity for binding to plasma protein or tissue and storage in such depots as fat and bone. There can also be differences between species with respect to plasma protein and tissue binding. These differences do not seem to be of major importance, but small mammals, such as mice, tend to bind substances less extensively than man. Research on such subjects as storage depots is needed.

Metabolic Differences. The primary organs that metabolize substances are the liver, the lungs, and the kidneys. With respect to hepatic metabolism--often considered to be the most important--small mammals generally metabolize substances more rapidly than large mammals, and herbivores more rapidly than carnivores. There are other interspecific differences; this is a field in which research is active.

Excretion And Reabsorption. There are few comparable data contrasting renal or hepatic excretory rates of substances in mice, rats, dogs, monkeys, and man. What data there are suggest that small mammals excrete compounds via the kidneys considerably more rapidly than large mammals. There are too few data to show systematic differences in hepatic excretion and the extent of reabsorption after hepatic excretion. Some substances are excreted through the hepatic system more rapidly by rodents than by dogs and other large animals. After excretion via the bile, reabsorption from the gastrointestinal tract may occur.

Differences in Receptor Sites. The action of cellular and intracellular membranes is also of concern. An unaltered substance or its metabolites, after distribution through the body, must pass through a variety of cellular and intracellular membranes to arrive at the site of action. Many of these membranes are of major importance in determining the ultimate toxicity of the substance. The blood-brain and blood-testicular barriers, for instance, effectively modulate the chemical composition of the fluid surrounding the central nervous system cells and the developing sperm cells. These barriers can also avert or delay the arrival of potentially-toxic compounds at the sites of action. Similarly, the placenta acts as a barrier between the maternal circulation and the developing fetus. In addition to the passive barriers, active-transport systems influence the movement of foreign organic compounds. This is a subject in which research is moving rapidly, and there is a great need for unified systematic theories on interspecific relationships. In general, if a substance reaches a site of action, the ultimate toxic reaction seems to be consistent among species. Development of a lesion will then follow this reaction. With some of the more serious manifestations, such as malignant change and mutagenic change, development of a lesion may depend on the extent of damage (e.g., severely damaged cells will die; generation time of the cells and rates of repair processes may be affected). For instance, the generation time of the rapidly proliferating cell population in the mouse is about half of that in man; therefore, cancer is likely to appear earlier in the mouse.

Design Of Laboratory Experiments On Animals

In designing assay procedures, the route and type of human exposure should be duplicated as closely as possible. Because it is may impossible, *a priori*, to determine specific susceptibility--and because man often often be the most susceptible species--more than one species of laboratory animal should be used. Short-lived species, such as rats and mice, should be chosen so that tests can be completed in a short time. Doses should be selected for long-term studies, so that exposure to the test substance causes little or no weight loss or shortening of life of animals under test. Sufficiently great exposures in animals need to be used to compensate for the possibly higher sensitivity of the human population and the uncertainties involved in extrapolation. However, the dose should not be so high as to produce acute toxic effects.

Some *logical and statistical considerations* in the design of animal experiments affect the extrapolation. Two major considerations are the *structure* of the experiments--how many doses, how spaced, inbred vs. outbred animals--and the *size* of the experiment--how many animals at each dose. If several doses are used, it may be possible to derive a dose-response relationship.

The faith that can be placed in an experimental result is also related to the size of the experiment. An experiment using 1,000 animals that shows no untoward effects at a high dose elicits much more confidence that there truly is no effect than does an experiment using 10 animals--even if it is conducted at a similarly high dose. A low response rate, 1 - 2%, is almost impossible to establish with an experiment that uses fewer than 500 animals. If the animals were spread out among several doses (some of which might give a 0.1% or 0.01% response), the number of animals needed to yield reliable figures would be in the hundreds of thousands.

Man is genetically heterogeneous and lives in a heterogeneous environment, so some experimenters feel more confident in their extrapolations if there are non-inbred animals in their experiments. Using non-inbred animals generally requires larger experiments to give reproducible results. One approach to this problem is to conduct many experiments, with several species, using inbred animals of each species.

Current Capabilities to Extrapolate

More and more patterns that are useful for extrapolation to man are being recognized and can be identified in the course of studying the pharmacological disposition of a substance. Most of the differences that have been observed suggest that man is more sensitive than the usual experimental animal, and this must be kept in mind in establishing permissible exposures for humans.

There are great difficulties in comparing the median animal to the not-so-average man. Man is not genetically homogeneous and is usually exposed to a much wider range of environmental conditions than the usual experimental animal. Differences in environmental factors are known to affect the toxicity of a substance. Differences in the genetic makeup of the individual can affect toxicity. These must be considered in the extrapolation of laboratory-animal toxicity data to man. We must predict for, and protect, the highly sensitive individual as well as the average or median person. Because of the multitude of man-made chemicals, the different habits and life styles of populations, and different eating habits, there is considerable variation in the intake of, or exposure to, environmental pollutants. These must also be considered in establishing permissible exposures to environmental agents.

Genetic differences among humans in the disposal of toxic agents have been studied only in the last decade. Such study has led to a new branch of pharmacology--pharmacokinetics. One example of the great variability was the observation that humans metabolized two tricyclic antidepressants at such widely different rates that the steady-state plasma concentration in a group of about 20 patients varied about 30-fold, and the half-time of disappearance from the plasma varied about 10-fold (Hammer and Sjoqvist, 1967). Also, the plasma concentration of isonicotinic acid hydrazide varies by a factor of almost 100 in different people, and this appears to be genetically controlled (Weber, 1976).

Many examples of unexpected toxic reactions to therapeutic agents have been found in the interactions of compounds that were normally safe when given alone (Conny and Burns, 1972). The likelihood that such synergistic interactions will occur with environmental pollutants is high. An early and striking example of unexpected synergistic interactions (hypertensive crisis) occurred in patients who, while taking monoamine oxidase inhibitors, ate cheese that contained bioactive amines. Although theoretically predictable, this effect had not been suggested in the literature before it was observed in patients.

Many sources of variation within the population of any species, such as age, sex, pregnancy, disease, can affect the response to foreign substances. An example is the difference between sexes in metabolism and toxicity of acetylsalicylic acid (Menguy _et al_., 1972).

In addition, such environmental variables as temperature, lighting, barometric pressure, humidity, and diet are known to affect the toxicity of environmental pollutants. For example, the oral absorption of pentobarbital is greatly altered by the prior ingestion of a small cheese sandwich. In this case, peak pentobarbital concentration in plasma is diminished by about 50%, and the duration of the effective plasma concentration is doubled (Bush _et al_., 1966).

Another difficulty in extrapolating from laboratory animals to man is the dose-duration problem. The lifetime of a mouse is about 2 years. A man's life expectancy is close to 70 years, and a woman's 5 years longer. Because man usually excretes compounds more slowly than mice, this means that man, with a very small daily intake, can develop a greater accumulation of a compound over many years of intake.

It is currently impossible to estimate the effects of all the genetic and environmental variability in the human population.

Although gains are being made in predicting effects of pollutants in man, safety testing must, for the present, continue to rely on the results of administration of high doses of substances in test animals to provide a basis for protecting the human population.

THRESHOLDS

Biological Considerations

Whether or not a particular effect follows a dose-response relationship that has a threshold depends entirely on the mechanism of the effect. Many effects have thresholds. For example, the gastrointestinal-radiation syndrome, acute drug toxicity, and radiation or drug control of some tumors, all have dose-response curves that show thresholds. The curves are sigmoid, and below a particular dose there is a zero probability of producing the effect, because the effect requires many independent events and will not occur until the number of such events exceeds some critical value. The gastrointestinal-radiation (or drug) syndrome is a case in point. An animal will not die until the number of intestinal crypt cells that have been killed exceeds a value that is critical to the integrity of the organ. Any radiation or drug dose that kills fewer cells than this critical number can be considered to be "safe" (at least for this one syndrome).

We are used to thinking in terms of thresholds and sigmoid dose-response curves. For example, if it costs $4,000 to buy an automobile, we do not imagine that we will have a 50% chance of buying the same vehicle for $2,000. If 100 aspirin tablets constitute a lethal dose, we do not calculate that we will have a 1% chance of dying if we swallow a single tablet. Because we know the mechanisms underlying these events, we expect thresholds to the dose-response curves, and indeed they are evident.

However, other effects may well *not* have threshold dose-effect relationships. If an effect can be caused by a single hit, a single molecule, or a single unit of exposure, then the effect in question cannot have a threshold in the dose-response relationship, no matter how unlikely it is that the single hit or event will produce the effect. Mutations in prokaryotic and eukaryotic cells can be caused by a single cluster of ion pairs which were produced by a beam of ionizing radiation. We would expect that mutations can be caused by a single molecule or perhaps group of molecules in proximity to the DNA. The necessary conclusion from this result is that the dose-response relationship for radiation and chemical mutagenesis cannot have a threshold and must be linear, at least at low doses.

It is one step further to correlate mutagenesis with carcinogenesis. Nevertheless, the evidence is strong that there is a close relationship between the two (McCann *et al.*, 1975; McCann and Ames, 1976; Ames, 1976; DHEW, 1977).

We therefore conclude that, if there is evidence that a particular carcinogen acts by directly causing a mutation in the DNA, it is likely that the dose-response curve for carcinogenesis will not show a threshold and will be linear with dose at low doses.

Consideration of the Dose-Response Relationship. In considering the possibility of thresholds for carcinogenesis, it is important to understand that there is no agent, chemical or physical, which induces in man a form of cancer that does not occur in the absence of that agent. In other words, when there is exposure to a material, we are not starting at an origin of zero cancers. Nor are we starting at an origin of zero carcinogenic agents in our environment. Thus, it is likely that any carcinogenic agent added to the environment will act by a particular mechanism on a particular cell population that is *already being acted on by the same mechanism to induce cancers*. This reasoning implies that the only way for a new carcinogen added to the environment to have a threshold in its dose-response curve would be if it were acting by a mechanism entirely different from that already being experienced by that tissue.

Examination of Experimental Dose-Response Curves. The most extensive information on carcinogenesis both in experimental animals and in humans is with ionizing radiation (BEIR, 1972). Although there is evidence implicating thresholds in some animal tissues (Walburg, 1974), thresholds have in general not been established for most tissues. If such thresholds exist, they occur at sufficiently low doses that it would require massive, expensive, and impracticable experiments to establish them. In view of the common finding--for example of a linear dose-response relationship (unaffected by dose-rate)--for cancer induction in animals by high LET radiation, it is unlikely that such thresholds exist. Linearity is not essential to the no-threshold argument since non-linear dose-response relationships do not necessarily imply the existence of thresholds.

Recent reviews by Barendsen (1975) and Brown (1976) suggest that the dose-response curve for mutation induction, the production of chromosome aberrations, and the induction of tumors in mammals is linear with low-LET radiation up to about 50 - 100 rads. Brown (1976) concluded from the available human data that this also applies to humans. Such findings argue against a threshold for ionizing radiation. Because many carcinogenic agents act like radiation in producing mutations, chromosome aberrations, and cell killing, we see this as an additional argument against the likelihood of thresholds in the dose-response curves of these agents.

Heterogeneity of the Population. The human population in the United States--the population we are trying to protect--is a large, diverse, and genetically heterogeneous group exposed to a variety of toxic agents. Genetic variability to carcinogenesis is well-documented (Strong, 1976), and it is also known that individuals who are deficient in immunological competence (for genetic or environmental reasons) are particularly susceptible to some forms of cancer (Cottier *et al.*, 1974). It seems, therefore, that even if we were to postulate an average threshold for a particular cancer induced by a particular agent, we would in practice need a series of thresholds for different individuals. It would be extremely difficult, in practice, to establish a single threshold.

We conclude from these arguments that, despite all the complexities of chemical carcinogenesis, thresholds in the dose-response relationships do not appear to exist for direct-acting carcinogens. If they do exist, they are unlikely to be detected and hence impossible to use. This means that there can be no totally "safe" exposure to a particular carcinogen, nor can the term "margin of safety" have any meaning. Any dose of a carcinogen must be considered to be associated with a risk, even if that risk is vanishingly small; estimates must be made of that risk.

Statistical Considerations

Many quantitative theories of carcinogenesis that have been proposed relate the frequency of detectable tumors to the intensity of exposure to the carcinogen. The purposes of these theories are twofold: to elucidate the mode of action of the carcinogen and the nature of the neoplastic change, and to estimate--from animal experimentation--the risk to human populations exposed to environmental concentrations of the carcinogen.

One of the earliest quantitative theories was that of Iverson and Arley (Arley and Iverson, 1952; Iverson and Arley, 1950). Their model was basically a one-step transition process occurring in a single cell. A cell starts out being "normal" and has some positive probability of being transformed to a cancer cell. Iverson and Arley assumed that this transition probability was a linear function of the amount of the carcinogen, the intercept of this linear function representing the background or spontaneous transition probability, as would be obtained if none of the carcinogen were present. After transition to a cancer cell, Iverson and Arley assumed, the growth of the clone could be represented by a pure birth process with a birth rate independent of the initial amount of the carcinogen. It was assumed that the clone would become a detectable tumor when it reached a given size. This model is commonly referred to as the one-hit model and implies that the expected number of tumors within a lifetime will depend only on the total dose and not on the pattern of exposure. The mathematical forms of a wide class of hit-theory models were given by Turner (1975), who developed the mathematical theory between the "hits" of an agent and the critical targets. Extensions that include both biological variation in the probability of a hit, and variation in the number of critical targets were also discussed.

Both Nordling (1953) and Stocks (1953) carried the Iverson-Arley model a step further when they proposed theories in which a single cell can generate a tumor only after it has undergone more than one change or mutation; these could be termed multievent theories of carcinogenesis. They assumed that, within some time-frame, the probabilities of transition from one state to the next were the same, and proportional to both time and the concentration of the carcinogen. Like Iverson and Arley, Nordling and Stocks assumed that the growth time of the tumor (after the last event had been attained) was either independent of the carcinogen or negligible. When the number of necessary changes is about 6 or 7, this model yields age-specific, cancer-incidence rates that are proportional to the fifth or sixth power of age and in this respect is consistent with human incidence data. However, the model also predicts that cancer incidence is proportional to the same high powers of the concentration of the carcinogen; this is not in agreement with the results of human and animal data. To avoid this discrepancy, Armitage and Doll (1954, 1961) modified the theory of Nordling and Stocks by assuming that the probabilities of transition between events were not all equal. They also assumed that only some of the transitional events depended on the carcinogen and that the remainder had a probability of spontaneous transition independent of the level of the carcinogen in question. With this modification, the model became consistent with both human and animal data that showed tumor incidence as related to either dose or the square of dose, but not higher powers. It should be noted that the theories of Nordling, Stocks, and Armitage and Doll are based on the concept that carcinogenesis has a single-cell origin, whereas a theory proposed by Fisher and Holloman (1951) is multicellular in concept. They proposed that a tissue of N cells must contain at least K cells that have undergone some transformation in order for a tumor to occur in the tissue. This theoretical approach leads to a model very similar in form to the multievent model. Other multievent theories have been proposed that incorporate the concepts of cell death or loss of ability to divide (Burch, 1960), and modifications that permit cells in intermediate stages to grow more rapidly than normal (Armitage and Doll, 1957).

Crump _et al._ (1976) discussed many of these models of carcinogenesis from the viewpoint of low-dose kinetics. They made two basic assumptions: that the cancer process is single-cell in origin, possibly with multiple steps between initiation and complete alteration, and that the growth period of the completely altered cell is basically independent of the degree of exposure. For direct carcinogenic processes, in which the agent or its metabolite acts at the cellular level to produce an irreversible change, they concluded that most models of carcinogenesis will be linear for low doses. In addition, they showed that, if it can be assumed that the environment contains carcinogens that act in conjunction with the test agent, then all the models thus far proposed will be linear for low doses.

In all these theories, the emphasis is mainly on the stochastic nature of the changes involved in the carcinogenic process. The role played by the carcinogen is considered to a much lesser degree. It is commonly assumed that transitional events in the process that are attributable to the carcinogen occur with probabilities proportional to the degree of exposure. This is undoubtedly a gross oversimplification of the actual process. The actual exposure is no doubt modified by absorption, distribution, metabolism, and excretion of the chemical substance, and the effective exposure should probably be the actual concentration of the carcinogen at and within the target cells. Other factors that may affect delivery of the carcinogen to intracellular compartments are membrane permeability and enzyme binding. Therefore, the "effective dose" may well be some complex function of the actual exposure and the biochemical and physiologic characteristics of the host. Most of these mathematical models incorporate the dose as it is actually administered in animal experiments or human exposure. The function relating administered dose to "effective dose," if it is not a simple case of proportionality, can have a profound effect on the dose-response relationship. As a simple example, consider a linear model relating dose (effective dose) to response. If the effective dose is proportional to the administered dose, then a linear model obtains for administered dose versus response. If the dose relationship is concave, which would obtain if the incremental increase in effective dose decreases with higher doses, then the relationship between administered dose and response would also be concave. Thus, the various dose-response curves that have been observed may not be indicative of different carcinogenic processes once the agent has reached the target cell, but rather may indicate different functions relating administered dose to effective dose. This problem will probably relate more to chemical carcinogenesis, as opposed to radiation-induced cancer.

Even if a normal cell has been transformed to some intermediate stage in the carcinogenic process, this would not necessarily mean that cancer must occur. Cell repair or recovery or some other response from the immune mechanisms may be sufficient to stop or reverse the process before the final stage is reached. In addition, the death of these transformed cells may occur before the process has a chance to continue toward the eventual cancer. This is one of the major arguments in favor of the existence of a threshold. However, if there is some probability that these recovery mechanisms will not complete their role before the occurrence of another event or transformation, then this type of threshold will not exist.

Thresholds may be considered from two viewpoints--an "actual" threshold, which is an exposure below which any carcinogenic response attributable to the specific agent is impossible, and a "practical" threshold, which is an exposure below which an attributable carcinogenic response is highly unlikely. In discussing carcinogenic thresholds, Mantel (1963) has argued that it is immaterial whether or not "actual" thresholds exist, and that one should consider the "practical" thresholds when estimating human risk. He has suggested procedures for extrapolating the results of animal experiments performed at necessarily high exposures to the lower exposures of humans; Mantel and Bryan (1961) stated that a risk of cancer of 1 in 10^8 could be thought of as a "practical" threshold and that efforts should be made to estimate exposures that would produce no more than this risk. Using mathematical models that relate the latency period (time between initiation of exposure and appearance of cancer) to the exposure, Jones (1976) suggested that a "practical" threshold could be defined as an exposure for which the latency period is beyond the normal lifespan.

Experimental or observational evidence of the existence of an "actual" threshold is usually presented in the form of a dose-response graph, in which the percentage of animals with tumors or the average number of tumors per animal is plotted against the dose of the carcinogen. Either the existence of doses which do not lead to an increase in tumor incidence over controls, or the extrapolation of such curves to low doses that apparently would result in no tumor increase, is cited as an indication of the existence of a threshold below which no response is possible. This type of evidence is an exercise in self-deception.

In the first case, the observation of no positive responses does not guarantee that the probability of response is actually zero. From a statistical viewpoint, zero responders out of a population of size N is consistent at the 5% significance level with an actual response probability between zero and approximately 3/N (e.g., when N=100 and zero responders are observed, the true probability of response may be as high as 3%).

In the second case, when an observed plot of dose against tumors is extrapolated downward to produce a no-effect dose, it is assumed that the observed dose-response relationship, usually linear, will obtain throughout the entire spectrum of doses and that one threshold exists for the entire population at risk. The assumption of linearity throughout the entire dose spectrum can easily lead to erroneous conclusions. For example, consider the true dose-response relationship shown by the dashed curve in Figure II-1.

This curve is convex, which would be consistent with a multievent theory of carcinogenesis in which more than one event is affected by the carcinogenic agent. This type of convex dose-response relationship, when sampled in an animal experiment over only a part of the dose range, could be thought to imply the existence of a threshold if simple linear extrapolation is used. If the animal experiment is performed at doses between A and B, one could conclude that a threshold exists at dose d_1; if the experiment is performed at doses between B and C, the conclusion could be that a higher threshold exists at dose d_2. In fact, for convex dose-response curves, if the experiment is performed over any range of doses that appears to produce linearity between dose and response, then simple linear extrapolation will always imply the existence of a threshold.

In addition, the assumption of one threshold is unrealistic. It is much more likely that, if thresholds do exist, not all members of the population have the same one. The human

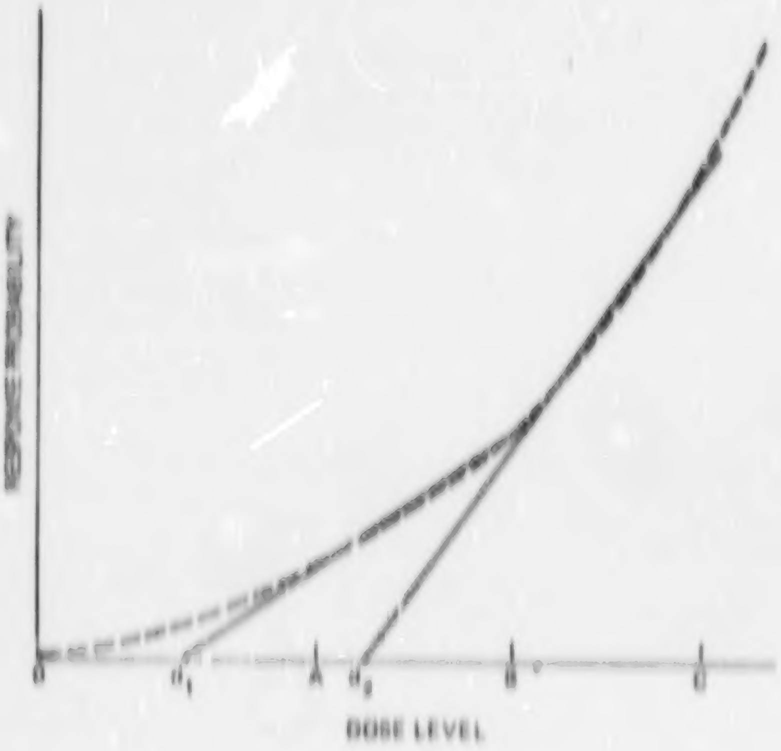

Figure II-1. Linear extrapolation from a convex dose-response curve.

population is a very diverse, genetically-heterogeneous group that is exposed in different degrees to a large variey of toxic agents. Many different disorders may affect the frequency of mutational events in specific tissues. Disorders characterized by chromosomal instability have been found to be predisposed to malignancy. Patients with xeroderma pigmentosum are highly susceptible to ultraviolet-induced skin cancer, and it has been found that they have deficient DNA repair mechanisms (Robbins *et al.*, 1974). In Bloom's syndrome, there is an immune deficiency and increased risk of leukemia and cancer of the colon (German, 1972). These systems may provide a model in which the risk of mutation and subsequent malignancy after exposure to an environmental carcinogen may be genetically determined. If malignancy is the result of a series of mutational events, then there must be subpopulations at various degrees of risks or with various thresholds for the carcinogenic agent. Therefore, the search for thresholds should not be a search for one specific no-effect dose applicable to all members of the population at risk; rather, the problem is to find many thresholds for each of the subgroups within the population.

This variability in thresholds or susceptibility to carcinogenic agents has been shown by Mantel *et al.* (1961) to induce an increased convexity in dose-response curves at low doses. They demonstrated that a linear dose-response curve with a fixed threshold will become convex at low exposures, if the individual thresholds are allowed to vary. Therefore, the extrapolation of observed dose-response curves, when the individual thresholds actually vary in the population, will, at best, simply lead to the average threshold of the population at risk. This estimate of the average threshold will have little practical utility, because many members of the population will have individual thresholds below this value. In addition, if we are willing to assume that threshold variability is the likely state in nature, then from a statistical viewpoint it is practically impossible to distinguish between mathematical models that hypothesize different thresholds and multievent models that hypothesize no thresholds, because the shapes of the two models can be very similar, and in some cases identical. For example, a one-hit, dose-response model with a threshold may be written as,

$$P(d|\lambda) = \begin{cases} 0 & d \le \lambda \\ 1 - e^{-\alpha(d-\lambda)} & d > \lambda \end{cases}$$

where P(d/λ) represents the dose-induced response rate at a dose level d and λ is the threshold below which no response can occur. If we assume that the population consists of individuals with different thresholds, and that these thresholds vary according to some probability distribution F(λ), then the variable-threshold model is simply the convolution of P(d/λ) with F(λ),

$$P(d) = \int_{\lambda} P(d|\lambda)dF(\lambda)$$

If, for computational simplicity, we choose F(λ) to be an exponential probability distribution, then this variable-threshold model takes the mathematical form,

$$P(d) = 1 - \frac{e^{-\alpha d} - \alpha/\beta e^{-\beta d}}{1 - \alpha/\beta}$$

The interesting aspect of this particular mathematical model is that, as the ratio α/β approaches unity, the model becomes

$$P(d) = 1 - (1 + \alpha d)e^{-\alpha d}$$

which is the mathematical form of a two-hit model for dose response.

Discrimination among these models on the basis of animal experiments is often impossible. These three models were fitted to data from an experiment by Terracini et al. (1967) in which dietary concentrations of dimethylnitrosamine (DMN) of 0-20 ppm were fed to female rats. The experiment was continued for 120 weeks, and the appearance of liver tumors was the response variable. The data are shown in the following table.

	DMN in diet (ppm)				
	0	2	5	10	20
Number responders/ at risk	0/29	0/18	4/62	2/5	15/23
Response rate	0.0%	0.0%	6.5%	40%	65%

The fixed-threshold and variable-threshold models, in addition to the two-hit model, all fit these data equally well. There is no statistical basis on which to prefer one over the others. Experimental results like these, although appearing to give evidence of a threshold, will provide no statistical evidence either in favor of, or opposed to, the existence of such thresholds. Therefore, statistical analysis of standard animal carcinogenicity experiments is not--and probably will never be--in a position to resolve the threshold question. There are too many "biologically reasonable" mathematical models, both implying and denying the existence of thresholds, that will fit the observed results.

The quantitative theories of carcinogenesis that have been proposed are all stochastic. They consider the probabilistic aspects of the occurrence of some series of events. If one is willing to assume that these events have transition probabilities that can be affected by the carcinogen (no matter how small the concentration) that the systems of distribution and metabolism have some chance of allowing some amount of the carcinogenic agent to reach the target cells (no matter how small the chance or the amount), and that the repair and recovery systems may not do a perfect job (no matter how small the chance that this will happen), then there will be no exposure that will have a zero probability of leading to a cancer.

In addition, when considering the possibility of carcinogenic thresholds, one should keep in mind that no agent has been found to induce a type of cancer that had not been previously described. It is possible, perhaps even likely, that many carcinogenic agents act by the same mechanism on the same target cells. This would imply that, inasmuch as there are many carcinogenic agents in our everyday environment, some additional carcinogen could act in a simple additive manner and thus, that any exposure would simply be added to the background. This means that for a population already being exposed to carcinogenic agents, any additional carcinogen would simply increase the expected tumor incidence in a continuous manner, no matter how low the exposure to the additional agent. Therefore, despite all the complexities of the mechanisms of chemical carcinogenesis, because of genetic variation among members of the population at risk and because statistical analysis cannot resolve the question one way or the other, the search for an "actual" carcinogenic threshold is probably fruitless, and any human exposure to a carcinogen should be considered to be associated with some risk, no matter how small that risk may be. The current mathematical models that relate exposure to attributable risk are, at best, extremely crude. Much work needs to be done to refine these theories.

HIGH-DOSE TO LOW-DOSE EXTRAPOLATION

Dose-Response Models

Animal experiments must be performed at doses high enough to produce tumors, and environmental concentrations must be low enough to produce few, if any, tumors. Therefore, to estimate the probability of response at dose levels outside the experimental range, it is necessary to make an assumption concerning the form of the dose-response relationship at low doses. Many quantitative theories of carcinogenesis have been proposed that relate the occurrence of detectable tumors both to the quantity and the duration of exposure to the carcinogenic agent. Most of these theories start with the premise that the carcinogenic process consists of one or more stages that occur at the cellular level, but that not all of these stages are related to the carcinogen. These stages may be cell mutations or other biological or chemical events and may be monocellular or multicellular in origin. The probability of transition to an event related to the carcinogen is assumed to be proportional to the exposure. The exact nature and causes of these events are largely unknown. However, the most important aspect of these quantitative theories of carcinogenesis is that most of them lead to mathematical models for which the probability of tumor occurrence is generally related to a polynomial function of dose. For low exposure, the region of most importance, they are all well-approximated by a simple linear function of dose (Iverson and Arley, 1950; Fisher and Holloman, 1951; Muller, 1951; Nordling, 1953). This class of dose-response models may be considered as models that are "linear at low dose." Other mathematical dose-response models have been proposed for this problem of extrapolating from high doses to low doses, the most notable being the log-probit method of Mantel and Bryan (1961). These types of models have little biological justification in what is known about the carcinogenic process. In addition, some require use of pre-selected parameter values that are chosen without regard to the particular experimental situation or results. The assumption of a particular dose-response model for extrapolation purposes should, at the very least, be consistent with current knowledge concerning the carcinogenic process.

All of these mathematical models that relate dose to response rate fall into two categories: dichotomous-response models, for which the important experimental fact is whether a particular condition, such as a malignant tumor, is present by a particular time (in chronic exposure experiments, this time is usually the animal's normal lifetime), and time-to-response models, for which the actual time to occurrence of the particular condition is known for each animal in the experiment. (For this type of model, the relationship of time, since initiation of exposure, to response can be ascertained along with the relationship of degree of exposure to response). Each type of model is completely specified, except for a number of unknown parameters that are to be estimated from the experimental results. These estimates and the functional form of the assumed dose-response model will provide an estimate of the risk attributable to the carcinogen at any desired dose.

In the dichotomous-response situation, the multievent theory of carcinogenesis proposed by Armitage and Doll (1961) leads to a mathematical model that relates the probability of response P(d) to the dose d by

$$P(d) = 1 - e^{-(\lambda_0 + \lambda_1 d + \lambda_2 d^2 + \ldots + \lambda_k d^k)}$$

where k represents the number of transitional events in the carcinogenic process that are related to the carcinogen under test and λ_0, λ_1, λ_2, ..., λ_k are unknown nonnegative parameters. As with the other "linear-at-low-dose" models, for small enough values of the exposure d, this dose-induced response rate will be approximately equal to $\lambda_1 d$ (assuming that λ_0 is the "background" rate). Therefore, in extrapolating from high dose levels to low doses, the risk attributable to the carcinogen, after correcting for background, will depend on the magnitude of the linear coefficient, λ_1.

When estimating risk with this model, the function is to be fitted to the experimental data by some such procedure as maximal likelihood (Guess and Crump, 1976). A point estimate of the attributable risk may be obtained from the model with the estimated parameters, but to incorporate the vagaries of random sampling, it would be prudent to include the upper-statistical confidence limit on this risk estimate.

Appropriateness of Data for Low-Dose Extrapolation

Before an actual high-dose to low-dose extrapolation is attempted, considerable attention must be given to the appropriateness of the experimental data to be used. The usual and often stated requirements for quality carcinogenesis bioassay procedures are necessary in order to avoid misleading risk estimates. Also, oral administration of the carcinogen is necessary, because we are concerned with estimating the risks associated with drinking water consumption. Another point is that the experimental animals should receive a lifetime administration of the carcinogen, because this corresponds to the human situation that we are attempting to estimate. It is not correct to assume that the tumor yield in a partial lifetime study will be equivalent to a lifetime study with the same total dose. This is a result of multistage models for carcinogenesis which assume that the cancer-incidence rate is proportional to a power (often 3 to 5) of duration of exposure. It may be possible to extrapolate from partial- to full-lifetime exposure, but it must be done on a sound biological basis.

Serious problems also develop from the actual data generated by the assay. These problems have to do with the need for some observed dose-response information. Often, the experimental doses are very high, and this produces cancers in almost all the experimentally-treated animals. This may be because the assay protocols are designed for the determination of carcinogenicity, and not for low-dose extrapolation and dose-response estimation. If all of the treated animals produce high incidences of tumors, one has no useful idea of where the dose-response curve actually is (particularly the lower convex portion of the curve). The true dose-response curve could be badly underestimated--even with the use of a straight-line extrapolation. Therefore, one must have some information concerning doses at which the tumor incidence is in the lower convex portion of the dose-response curve.

Although low-dose extrapolations are out of the question for high-incidence data, one can calculate some relative dose information that can be used in expressing concerns over possible adverse health effects. Essentially, the exposure which is of interest in man is converted to the equivalent dose in animal study. The ratio of the lowest experimental dose to the equivalent human dose is then calculated. Clearly, the closer this ratio is to 1:1, the greater the concern.

One final point should be made. The extrapolations should generally be conducted on total tumor yields, as opposed to specific tumor types, because we wish to estimate total cancer risk in man, not cancer risk in man for a specific tumor type; if, of course, the total-tumor data are too noisy, then specific types will be required, and specific types may also be of interest for comparisons with human data.

INTERACTIONS

A major concern in carcinogenesis, and in assessing the safety of a single material, lies in the answer to the question "How does this material behave in the presence (or absence) of other materials?" Is the response to two materials the sum of the individual responses? Do the effects come about as if doses were added? Is there more (or less) than additivity? Do some things cancel each other out?

Some experience in man begins to give answers to these questions. The few known interactive effects in man are related to exposures to cigarette smoking combined with some other exposure--industrial or social. These are the combined effects of: cigarette smoking; (inhaled) asbestos exposure (Selikoff, 1968); inhalation of radon, as in the case of the uranium miners (Lundin, 1971); and the use of alcohol (Rothman, 1972). All of these show risks that are more than additive. For example, the heavy smoker has about two times the risk of the non-smoker of dying of cancer of the oral cavity. The heavy drinker-heavy smoker has about 15 times the risk of the non-smoker, non-drinker. Reports from Great Britain support the idea that the recent reduction in male lung cancer mortality, particularly in Greater London, with small changes in cigarette smoking and large changes in air pollution have come about through an interaction between urban air pollution, and cigarette smoking. (Lawther, 1976).

Animal experimentation (Berenblum, 1947) showed an initiator-promoter effect, in which each material applied separately (to the skin of test animals) had little or no carcinogenic effect. The two materials, applied in the appropriate sequences, however, were highly carcinogenic. At one time it appeared that there were materials that were purely initiators (they started the process) and there were others that were purely promoters (they moved the process along to frank cancer). More recent views suggest that some materials are both initiators and promoters, sometimes acting as one, sometimes as the other, and sometimes as both, giving a "complete" carcinogen. (Berenblum, 1974)

The exact nature of initiation and promotion is not clear, and there is evidence that there is initiation-inhibition as well. (Falk, 1971) Thus, it may be possible that the carcinogenic effect of one material may be inhibited by another material. Genetic susceptibility can be included in the initiator-promoter model. Increased genetic susceptibility may be providing an initiator step, while increased genetic resistance will look like initiation-inhibition. Some cases of this are rather obvious in man. For example, ultraviolet radiation leads to skin cancer, mostly in people with fair skin, light hair and light eyes (Urbach, 1969). We know that these latter characteristics are genetically determined and so we speak of genetic susceptibility. Dark-skinned, dark-eyed, dark-haired people have genetic resistance. It is not nearly so obvious, nor do we know the genetics involved, in the case of the higher susceptibility to breast cancer among women from "breast cancer families." Women who come from families where a mother, sisters, or the fathers' sister(s) have breast cancer are at higher (twofold to threefold) risk than women from non-breast cancer families (Lilienfeld, 1963). There is nothing so obvious nor so directly involved as skin pigmentation to give any leads here.

A possibly unifying concept concerning interactions is the "fertile ground" idea. If the carcinogen falls on fertile ground, it will lead to cancer growth. If not, no cancer. This implies a whole set of questions about the nature of the "fertile ground." Some animal experimentation seems to imply that, with the induction of mammary tumors in the rat by hormonal stimulation, the ground is made fertile by earlier viral infection (Shellabarger, 1976). Similar results seem to be shown in the induction of (mouse) cancers through the combination of malaria infection and exposure to the Moloney mouse-tumor virus (Wedderburn, 1970). A similar mechanism has been suggested for the development of Burkitt's lymphoma in children in East Africa, in malaria infection associated with appropriate viral infection (Morrow, 1976).

Another way that fertile ground can be left is through a deficiency interaction. Here, the absence of some element, material, vitamin etc., may reduce the ability of the organism's repair mechanism, so that a level of a carcinogen that produces little or no cancer in a population capable of repair produces much cancer in a population of repair-deficient individuals. An example is the high cancer rate in Xeroderma pigmentosum patients--people with a low ability for DNA repair (Robbins, 1974; Cleaver, 1968). A similar mechanism (on a grosser level) may be involved in the high incidence of cancer of the esophagus in central Asians whose bread and tea diet may not contain the elements necessary to permit repair of tissue affected by low levels of a nonidentified carcinogen (Mahboubi, 1973).

Much of the classical toxicology of dose-response assumes a distribution of sensitivities in a population. Individuals whose personal threshold is lower than the dose given will respond by developing the condition. Individuals with higher thresholds will not. The existence of dose-response curves that are not simple step-functions (zero response at dose d, 100% response at dose d+e, where e is some very small increment of dose) suggests these host-environment interactions. Individuals with high glutathione and sulfhydryl levels in their livers may be able to "handle" (to metabolize to some non-active form) toxicants much better than individuals with low levels (Gillette, 1974).

According to Brown (1975), no carcinogen has been found to induce a cancer of an entirely new, never-before-seen histological type. A possible explanation of this is that many carcinogenic agents or processes act through the same ultimate mechanism in the same target cells. He writes "...since there are many carcinogenic agents in our...environment, (a new) carcinogen could act in a simple, additive manner and thus any level of exposure would...be added to the...existing background." This kind of interaction phenomenon has important implications for the models used in extrapolation. It implies that these models may not assume any threshold for a new material in a world already populated by many carcinogens. It also leads to the conclusion by Crump *et al.* (1976) that "all models of carcinogenesis thus far proposed will be linear at low doses."

The discovery of interactions will require more sophisticated experimental techniques than are now being used. Testing combinations of materials multiplies the number of tests that must be done (100 materials, tested two at a time, require 100 x 99/2! tests;--tested three at a time require 100 x 99 x 98/3! tests). New techniques for multiple testing will have to be developed. Uncovering deficiency interactions will require an entirely new and different set of approaches.

SUMMARY AND CONCLUSIONS

Large populations are repeatedly exposed to potentially-toxic contaminants in the drinking water--in minute amounts over many months or years, or over whole lifetimes. Delayed, essentially irreversible, effects can occur. Methods and criteria of classical, conventional toxicology do not offer reliable means for assessing long-term toxic effects such as carcinogenesis in man by extrapolation from animal data; hence, novel considerations have to be applied in assessing risk. The insidious effect of chronic exposure to low doses of toxic agents is difficult to recognize, because there are few, if any, early warning signs and, when signs are ultimately observed, they often imply irreversible effects. For example, cancer induction in experimental animals, even with the most potent carcinogenic chemicals, requires at least several months and in many instances a whole lifetime. There are as yet no easy, straightforward methods for extrapolation of even chronic-exposure experimental data to calculate risks to large human populations. Teratogenic effects are easier to establish by animal experimentation, but there are similar uncertainties in extrapolating to human populations. Mutagenic effects are difficult to assess experimentally in mammals, and such effects are particularly insidious, in that they appear only in later generations.

For assessment of acute toxicity, various characteristics--such as LD_{10}, LD_{50}, and maximal tolerated dose--are generally quantitatively similar among most animals. On the basis of dose-per-unit of body surface, toxic effects in man are in the same range as those in experimental animals, such as mouse, rat, hamster, dog, and monkey. On a body-weight basis, man is generally more vulnerable than the experimental animal, probably by a factor of 6-12. From comparative studies, general principles have emerged that the absorption, metabolism, and excretion of various drugs are slower, dose-for-dose, in man, and that there is greater retention of such drugs; also, higher concentrations occur in body fluids and tissues in man than in small mammals. With an awareness of these quantitative differences, appropriate safety factors can be applied to calculate relatively safe therapeutic dosages for man. These methods and principles of classical toxicology are useful for assessing toxic effects that are reversible and nonprogressive. They are much less useful in dealing with the problems of chronic irreversible toxicity or the effects of long-term exposure. This subject has not been considered widely in the past.

We outline here some principles that underlie all efforts toward this goal, principles we believe are supported by existing knowledge. From the review of available information, two major questions emerge--"What types of experimental-assay procedures are required for a valid assessment of chronic toxicity of chemicals in experimental animals?" and "How can such data be extrapolated to estimate risks in humans?" In dealing with these questions, we shall restrict our recommendations to one specific risk--namely, cancer--with the understanding that the same considerations will apply at least partially to the problems of mutagenesis and teratogenesis. Furthermore, we consider only carcinogens whose mechanisms involve somatic mutations.

Principle 1

Effects in animals, properly qualified, are applicable to man. This premise underlies all of experimental biology and medicine, but because it is continually questioned with regard to human cancer, it is desirable to point out that cancer in men and animals is strikingly similar. Virtually every form of human cancer has an experimental counterpart, and every form of multicellular organism is subject to cancer, including insects, fish, and plants. Although there are differences in susceptibility between different animal species, between different strains of the same species, and between individuals of the same strain, carcinogenic chemicals will affect most test species; and there are large bodies of experimental data which indicate that exposures that are carcinogenic to animals are likely to be carcinogenic to man, and vice versa.

Evidence that circumstances leading to cancer induction in humans are also applicable to experimental animals stems from the very first observation of chemical carcinogenesis--the appearance of cancer of the scrotum in chimney sweeps, observed by the British surgeon, Percival Pott, in 1775. It was not until modern times that a substance implicated in human cancer was found to be carcinogenic in animals, when the Japanese scientists, K. Yamagiwa and K. Ichikawa, found in 1915 that extracts from coal tar caused cancer when applied to the skin of experimental animals. Many pure carcinogenic chemicals have since been isolated from a wide variety of "tars" derived from incomplete combustion of organic matter, such as coal, wood, and tobacco. There is little doubt that these and other chemicals, alone or in combination, are responsible for the greatly-increased incidence of lung cancer among smokers. With the possible exception of arsenic and benzene, all known carcinogens in man are also carcinogenic in some species, although not in all that were tested. All carcinogens in animals are not known to be carcinogenic in man.

Principle 2

Methods do not now exist to establish a threshold for long-term effects of toxic agents. With respect to carcinogenesis, it seems plausible at first thought, and it has often been argued, that a threshold must exist below which even the most toxic substance would be harmless. Unfortunately, a threshold cannot be established experimentally that is applicable to a total population. A time-honored practice of classical toxicology is the establishment of maximal tolerated (no-effect) doses in humans based on finding a no-observed-adverse-effect dose in chronic experiments in animals, and to divide this dose by a "safety factor" of, say, 100, to designate a "safe" dose in humans. There is no scientific basis for such estimations of safe doses in connection with carcinogenesis. For example, even if no tumors are obtained in an assay of 100 animals, this means only that at a 95% confidence level, the true incidence of cancer in this group of animals is less than 3% Even if we were to carry out the formidable task of using 1,000 animals for assay and no tumors appeared, we could only be 95% sure that the true incidence were less than 0.3%. Obviously, 0.3% is a very high risk for a large human population.

In fact, there are no valid reasons to assume that false-negative results of carcinogenicity tests are much less frequent than false-positive ones. To dismiss all compounds that did not induce tumors in one or two mouse and rat experiments as noncarcinogenic is wrong. Labeling as "carcinogens" all substances that gave rise to increased incidence of tumors is justified only if there is conclusive evidence of a causal relationship. The "relative risk" of compounds that are not found to induce tumors in animal experiments must also be considered. But this requires evaluation of data other than those collected in chronic toxicity studies on rodents.

Experimental procedures of bioassay in which even relatively large numbers of animals are used are likely to detect only strong carcinogens. Even when negative results are obtained in such bioassays, it is not certain that the agent tested is unequivocally safe for man. Therefore, we must accept and use possibly fallible measures of estimating hazard to man. This reasoning leads us to the statement of Principles 3 and 4.

Principle 3

The exposure of experimental animals to toxic agents in high doses is a necessary and valid method of discovering possible carcinogenic hazards in man. The most commonly expressed objection to regulatory decisions based on carcinogenesis observed in animal experiments is that the high dosages to which animals are exposed have no relevance in assessment of human risks. It is therefore important to clarify this crucial issue. It should be recognized that--in general--only dosages that are high in relation to expected human exposures must be given to animals under the experimental conditions that are used. In setting up model experimental systems, we have no choice but to use numbers of animals that are small, in comparison with exposed human populations.

Even an incidence of cancer as low as 10% in a group of experimental animals, which would represent a lower limit of reproducibility, is exceedingly high, when translated to numbers of people. For example, an incidence as low as 0.01% would represent 20,000 people in a total population of 200 million and would be considered unacceptably high, even if benefits were sizable. Detecting such a low incidence in experimental animals directly would require hundreds of thousands of animals. For this reason, we have no choice but to give large doses to relatively small experimental groups and then use biologically reasonable models in extrapolating the results to estimate risk at low doses. Several methods of making such calculations have been considered and used, but we think that the best method available to us today is to assume "no threshold" and a direct proportionality between the size of the dose and the incidence of tumors. However, it is important to recognize that such calculations may give too small a risk. The actual risk to humans might be even greater over a human lifetime, because it is about 35 times that of a mouse; and there is evidence that the risk of cancer increases rapidly with the length of exposure. Moreover, experimental assays are conducted under controlled dietary and environmental conditions with genetically homogeneous animals, whereas humans live under diverse conditions are genetically heterogeneous, and are likely to include subpopulations of unusual susceptibility.

It should be emphasized that these general considerations give only a *minimal* estimate of human risk; moreover, they do not take into consideration species differences in susceptibility. For example, beta-naphthylamine is well established as a human carcinogen on the basis of epidemiologic studies of occupationally-exposed workers, whereas experiments have not shown it to be carcinogenic in, for example, the hamster, which is relatively resistant.

Not all substances that cause a given incidence of cancer in the rat are equally carcinogenic for man. This means that chronic-toxicity studies, which are imperfect assay systems for carcinogenicity testing, should not be used as the sole criterion in the assessment of risk.

Principle 8

Material should be assessed in terms of human risk, rather than as "safe" or "unsafe." The limitations of the current experimental techniques do not allow us to establish safe doses, but with the help of statistical methods we may be able to estimate an upper limit of the risk to human populations. To calculate such a risk, we need the following: data to estimate population exposure; a valid, accurate, precise, and reproducible assay procedure in animals; and appropriate statistical methods. Several general guidelines may be presented. First, no rigid, generally-applicable procedure can be recommended for testing all toxic agents; substances differ too much in their overall effects. The design of appropriate assays will ultimately have to be left to the well-informed judgment of expert investigators. If substances that affect large populations are found to be carcinogenic, experiments of much wider scope may have to be conducted to obtain more detailed information on their possible effects in humans. As a pragmatic guideline, it would be desirable to test a compound for carcinogenicity in at least two species, such as the mouse and the rat, and the strains of animals used should have a rather low incidence of spontaneous tumors under the conditions of the test. It is important to include "positive" controls, with known carcinogens, under the same conditions used for the test animals. This has been a point of considerable controversy.

Experiments should be conducted over as much of the lifetime of the experimental animal as possible. The highest dose should be the maximum that is tolerated without shortening the lifespan through causes other than cancer. Every animal, whether it dies during the exposure period, or is sacrificed at the end of the experiment, should be examined grossly and microscopically, and all toxic effects (not only cancer) should be noted.

Risk constitutes but half the essential comparison that should be made in the assessments of human hazard; the other half is--benefit to the exposed population of the agent for which hazard has been identified. Decisions cannot involve merely the risk. Statements of benefits should include the nature, extent, and recipient of the benefit. Technology has always been associated with some risk. But the acceptability of risk should depend on the specific benefits derived, the nature of the population exposed, and the availability of practical alternatives.

It is not possible to guarantee a risk-free society; nor is a risk-free society necessarily the best society. It is often necessary to accept the risks of chemicals--such as drugs and pesticides--when the benefits warrant their use. Risks imposed on persons who gain no benefits are generally not acceptable. Personal choice and personal values enter into the risk-benefit comparison. For major benefits--for example, in the treatment of otherwise incurable or incapacitating diseases--much higher risks are allowable than otherwise. An important principle in risk-benefit assessment is that each person must be allowed the widest possible choice--supported by full information on risks, as well as benefits--so that he might be able to make intelligent choices.

The benefit portion of the equation should be well defined by knowledgeable experts. It is important, therefore, that the benefit-risk comparisons be established with the active cooperation of people who are qualified to assess the usefulness of a substance and the consequences to those in need of it, as well as to the population at large.

Finally, mankind is already exposed to many carcinogens whose presence in the environment cannot be easily controlled. In view of the nature of cancer, the long, latent period of its development, and the irreversibility of chemical carcinogenesis, it would be highly improper to expose the general population to an increased risk if the benefits were small, questionable, or restricted to limited segments of the population. Such benefit-risk considerations not only must be based on scientific facts but also must be ethical, with as broad a population base as possible used in the decision-making process.

Principles To Be Used For Noncarcinogens and Nonmutagens

The nature and reversibility of the toxic effect must be considered.

For carcinogens that are not shown to be mutagens, some sort of extrapolation must be postulated.

For noncarcinogens for which it seems likely that there are thresholds for toxic effects, the acceptable dose should be below the threshold. If a threshold cannot be shown, the acceptable dose must be related to the data from animal experimentation and consideration of the seriousness of the toxic effects, as well as the likelihood and ease of reversibility, the variability of the sensitivity of the exposed population, and the economic and health-related importance of the material.

Research Recommendations

Research must be supported to develop an understanding of the mechanisms by which water pollutants produce toxic effects. This includes pharmacokinetics, toxication-detoxication mechanisms and biochemical and pathological mechanisms of action.

Estimates of margin of safety can be made more precisely and rationally as more is known about what happens to a chemical in the body and what the chemical or its metabolites do to the body. The results of such research also are necessary to develop rapid, inexpensive, accurate screening tests for various critical forms of toxicity.

It is recognized that much of this research is going on, but the Committee is convinced that more must be done. In protecting the population of the United States from environmental pollution there is no more important or potentially productive effort than the support of this kind of research. Since these studies are long-term in nature and must be closely coupled to basic biomedical research, they should be supported primarily by research rather than regulatory agencies.

There are many research needs in the field of chronic disease epidemiology. Manpower is in critically short supply. There are critical problems of data resources.

Research on statistical methods and mathematical models for estimating low dose effects should be encouraged. Statistical work is practically nonexistent for effects other than carcinogenesis. Although a considerable effort has been expended on dose-response estimation for carcinogenesis, very little has been done on species variability and susceptible subgroups. This area could at least be studied from an empirical standpoint so that there would be a better understanding of the precision of low dose risk estimates.

These recommendations are summarized below:

1. Studies of the physiological and biochemical mechanisms by which the toxic substances in water produce their effects.
2. Development of rapid, inexpensive, and precise tests to identify substances that may produce important toxic effects at low doses and dose rates.
3. Epidemiological studies of chronic disease.
4. Research on statistical methods and analytical models for describing and estimating the effects of long exposure to low doses of toxic substances. Studies should not be limited to carcinogenesis and should consider, also, differences between species, and particularly sensitive subgroups in the population.

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CHAPTER III
WATERBORNE MICROORGANISMS

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Chapter III
MICROBIOLOGY OF DRINKING WATER

The principal microbiological contaminants found in drinking water of the United States are bacteria, viruses, and pathogenic protozoa. Each is considered in a separate section of this chapter. Helminths are included along with the protozoa. Little information is available on mycoplasma, pathogenic yeast, and pathogenic fungi in drinking water. Microbiological contaminants, such as fungi and algae, do not seem to be important causes of waterborne disease, although they are sometimes associated with undesirable tastes and odors.

Epidemiology

The average annual number of waterborne-disease outbreaks in the United States reported since 1938 is shown in Figure III-1 (Center for Disease Control, 1976b). There was a decrease in the number of outbreaks during the late 1930's and 1940's, but this trend was reversed in the early 1950's. There has been a pronounced increase in the outbreaks reported by the Center for Disease Control (CDC) in Atlanta, Georgia, since 1971. The reason for this apparent increase is not entirely clear, but it could be either the result of improved reporting or an overloading of our treatment plants with source water of increasingly lower quality. Since 1971, the CDC, the Environmental Protection Agency (EPA), state epidemiologists, and engineers in state water-supply surveillance agencies have cooperated in the annual reporting of outbreaks. The purposes of such reports are to control disease by identifying contaminated water sources and purifying them, and to increase knowledge of disease causation. The roles of many microbial agents, including for example, *Yersinia enterocolitica* and mycoplasma, remain to be clarified.

The most important waterborne infectious diseases that occurred in 1971-1974 are listed in Table III-1. The etiologic agent was determined in only 53% of 99 disease outbreaks that involved 16,950 cases (Craun *et al.*, 1976). The remainder were characterized as "acute gastrointestinal illness of unknown etiology."

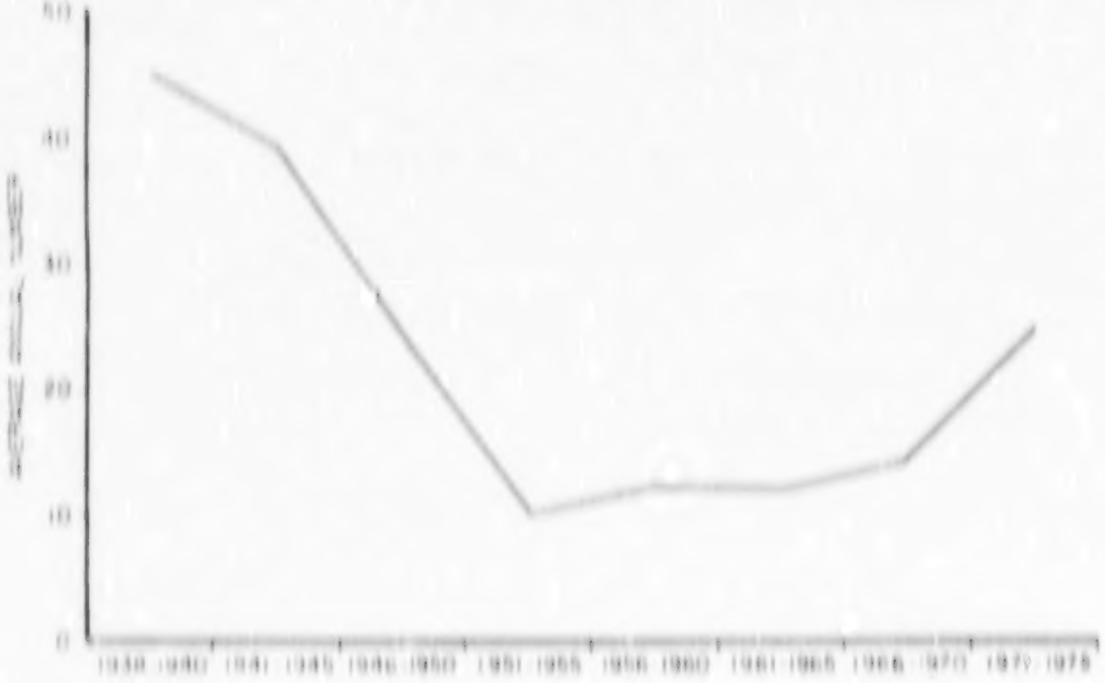

Figure III-1. Average Annual Number Waterborne Disease Outbreaks 1938-1975

TABLE III-1

Etiology Of Waterborne Outbreaks And Cases 1971-1974

Disease	Outbreaks	Cases
Gastroenteritis	46	7992
Giardiasis	12	5127
Shigellosis	13	2747
Chemical poisoning	9	474
Hepatitis-A	13	351
Typhoid	4	222
Salmonellosis	2	37
TOTAL	99	16950

Shigellosis was the most commonly identified bacterial disease (2,747 cases) in 1971-1974. Most of the cases were associated with non-municipal water systems. Four typhoid fever outbreaks affected 222 people and involved semipublic and individual water systems.

In 1974, 28 waterborne-disease outbreaks, comprising 8,413 cases were reported to the Center for Disease Control (1976a). The largest was an outbreak of giardiasis that occurred in Rome, N. Y., with an estimated 4,800 cases. The second largest involved about 1,200 cases caused by *Shigella sonnei*. In the third largest, which involved 615 cases of acute gastrointestinal illness, the etiologic agent was not definitely determined, but *Yersinia enterocolitica* was suspected. The fourth largest was caused by *Shigella sonnei* and involved 600 persons. Nineteen states reported at least one outbreak. Craun *et al.*, (1976) stated that "this probably reflects the level of interest in investigating and reporting in different states rather than the true magnitude of the problem within the state."

Semi-public water systems were associated with 55% of the outbreaks and accounted for 32% of the total cases in 1971-1974. Municipal systems accounted for 31% of the outbreaks, but 67% of the cases. Individual systems accounted for 14% of the outbreaks and only 1% of the cases, but outbreaks associated with individual systems probably are under-reported, as opposed to those associated with municipal and semi-public systems.

Deficiencies in treatment, and contamination of groundwater were responsible for a majority of the outbreaks (65%) and cases (63%) in 1971-1974. Inadequate or interrupted chlorination was involved in 31% of the outbreaks and 44% of the cases.

Craun *et al*. (1976) have drawn attention to the large number of waterborne disease outbreaks involving travelers. In 1971-1974, 49 (72%) of the 68 outbreaks that occurred in connection with semi-public and individual systems affected travelers, campers, visitors to recreational areas, or restaurant patrons; and (86%) of the 49 outbreaks occurred during April-September.

Outbreaks on cruise ships are excluded from the above tabulations, but they are of interest and should be mentioned because they involve the traveling public. For example, in June 1973, about 90% of 655 passengers and 35% of 299 crew were affected by an outbreak of acute gastroenteritis. An epidemiological investigation identified *Shigella flexneri* type 6 among early cases, and contaminated water and ice aboard the ship were implicated as vehicles of transmission (Center for Disease Control, 1973). In 1975, outbreaks of diarrhea on 8 ships affected between 9% and 61% of the passengers. In most of these outbreaks the causal agents and vehicles of transmission were unknown; water was identified as the vehicle in one of them (Center for Disease Control 1976b).

In 1975, 24 waterborne disease outbreaks involving 10,879 cases were reported to the Center for Disease Control (1976b). No etiologic agent was found for the two largest outbreaks (Sewickley, Pennsylvania--5,000 cases and Sellersburg, Indiana--1,400 cases). The third largest outbreak, involving over 1,000 persons, occurred at Crater Lake National Park, Oregon. Enterotoxigenic *E. coli* was isolated from residents of the park who became ill, and from the park's water supply.

Seventeen of the 24 outbreaks and about 92% of the cases reported to CDC were designated as "acute gastrointestinal illness." This category includes cases characterized by gastrointestinal symptoms for which no specific etiologic agent was identified. Cases resulting from water treatment deficiencies (60%) or deficiencies in the water distribution system (20%) accounted for almost 80% of the total cases in 1975. As in the past, most of the cases occurred in the spring and summer.

The reported numbers of outbreaks and illnesses represent only a portion of the true totals. Craun et al. (1976) called attention to the outbreak at Richmond Heights, Florida, in 1974 as an example of why good disease surveillance is necessary and of the way in which many illnesses may go unnoticed. Initially, only 12 cases of shigellosis in this outbreak were recognized by authorities. An epidemiologic investigation revealed that approximately 1,200 illnesses actually occurred. This large outbreak might not have been detected if local health authorities had not been conducting shigellosis surveillance. In another outbreak, some 1,400 residents of Sellersburg, Indiana (40% of the town's population) experienced gastroenteritis. The high attack rate, rapid onset of the outbreak, review of water sampling data, and the town-wide survey, suggested that the illness was waterborne, but no bacterial or viral pathogens or chemical toxins were found in the town water supply. When requested by a State health department, the CDC and EPA can offer epidemiologic assistance and expertise in engineering and environmental aspects of water purification. Requests for assistance often depend on the interest of a particular health department or person. Until improved detection and reporting systems are in use, the available epidemiological data will represent only a small fraction of the waterborne-disease problems in this country.

Bacteria

The principal bacterial agents * that have been shown to cause human intestinal disease associated with drinking water are: *Salmonella typhi*, typhoid fever; *Salmonella paratyphi-A*, paratyphoid fever; *Salmonella* (other species and a great number of serotypes); salmonellosis, enteric fever; *Shigella dysenteriae*, *S. flexneri*, and *S. sonnei*, bacillary dysentery; *Vibrio cholerae*, cholera; *Leptospira sp.*, leptospirosis; *Yersinia enterocolitica*, gastroenteritis; *Francisella tularensis*, tularemia; *Escherichia coli* (specific enteropathogenic strains), gastroenteritis; and *Pseudomonas aeruginosa*, various infections. Several other organisms have been associated with gastroenteritis, such as those in other genera of the Enterobacteriaceae: *Edwardsiella*, *Proteus*, *Serratia*, and *Bacillus*.

Number Of Cells Required To Infect. In attempting to assess the hazards in drinking water, it is important to know how many viable pathogenic cells are necessary to initiate an infection. McCullough and Eisele (1951, a,b,c,d) found that a dose of 10^5-10^8 salmonellae per person was necessary for most strains, although 10^5 cells of some strains could infect. More recent studies by Dupont, Hornick, and associates on selected enteric bacterial pathogens are summarized in Table III-2. Some enteric pathogens are highly virulent, causing infection when relatively few cells are administered (e.g., *Shigella flexneri* and *S. dysenteriae*), whereas others require large numbers to infect (e.g., *Salmonella typhosa* and *Vibrio cholerae*).

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*Nomenclature in this report follows the 8th edition of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

TABLE III-2
Infective Doses For Man Of Bacterial Enteric Pathogens

Subjects Infected/Total Tested

Enteric Pathogen Dose: Viable Cells	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9
Shigella dysenteriae									
Strain M131	1/10	2/4	7/10	5/6					
Strain A-1		1/4		2/6					
Shigella flexneri									
Strain 2A*		6/33*	33/49**	66/87	15/26				
Strain 2A**				1/4	3/4	7/8	13/19	7/8	
Salmonella typhi									
Strain Quailes			0/14		32/116		16/32	7/8	
Vibrio cholerae									
Strain Inaba									
With $NaHCO_3$				11/13		45/52		2/2	
No $NaHCO_3$				0/2		0/4	0/8	2/4	1/2
Enteropathogenic *E. coli*									
Strain 4608				0/5		0/5		4/8	

Data from: *Shigella dysenteriae*: Levine, *et al.*, 1973
Shigella flexneri: Dupont, *et al.*, 1972b*
Dupont, *et al.*, 1969**

Salmonella typhi: Hornick, *et al.*, 1970
Vibrio cholerae: Cash, *et al.*, 1974

Enteropathogenic *E. coli*: Dupont, *et al.*, 1971

* Dose: 1.8 x 10^2

** Dose: 5 x 10^3

Virulence is a genetic trait and can vary markedly from strain to strain (Meynell, 1961). Phenotypic variation in virulence can occur within a given clone. A small percentage of the cells in a population may be unusually virulent (Meynell, 1961; Meynell and Meynell, 1965). Thus, it does not always follow because large numbers of cells are required for infection in feeding trials, that large numbers in drinking water are necessary to cause infection. Some few individuals may become infected by small numbers of unusually virulent cells. Recent evidence also indicates the possibility of genetic transfer of virulence from invading microbes into the resident intestinal population, providing another means by which small numbers of organisms might initiate a disease state.

The consequences of an increasing prevalence in livestock and their excreta of coliform organisms containing infectious plasmids and giving rise to clinical conditions were not examined in detail because of time constraints and their lack of immediate relevance to standard setting. Similarly, the consequences of adding antibiotics to animal and poultry feed and the enhanced hazards of spreading drug resistant organisms were not examined.

The infecting dose also varies with the age and general health of the host population (MacKenzie and Livingstone, 1968). Infants and the aged may be particularly susceptible. Previous exposure to a given pathogen is important, in that coproantibodies may prevent infection with a strain that is generally present in the population, whereas a new serotype introduced into the water supply may present an increased hazard.

Not all strains of *Shigella* are highly virulent. Shaughnessy *et al.* (1946) determined infecting doses of four strains of *Shigella* while studying immunization in volunteers. They found that infectivity in mice could not be directly correlated with infectivity in humans and that doses of 10^9 organisms or higher were needed to produce human infection. In their extensive studies to develop a *Shigella* vaccine, Hornick, DuPont, and associates observed the infective dose for several strains. With *S. flexneri* 2A, 10 of 39 volunteers became ill from a dose of 10^4-10^8 organisms (DuPont *et al.*, 1969). They showed that *Shigella* must penetrate the intestinal mucosa to produce symptoms of classic dysentery and that addition of bicarbonate facilitated this process. Attenuated vaccine strains of *S. flexneri* 2a could be safely administered orally in doses of 10^{10} organisms or higher (DuPont *et al.*, 1972a). A virulent strain could cause symptoms in doses of as few as 180 organisms (DuPont *et al.*, 1972b). With *Shigella dysenteriae* 1 (Shiga strain)--an organism that has two pathogenic modes, invasiveness and enterotoxin elaboration--the infecting dose in man was shown to be as low as 10 organisms (TABLE III-2).

With such high infectivity of *Shigella*, why aren't waterborne outbreaks more common? One possibility is that *Shigella* survives poorly in water. Wang *et al.* (1956) pointed out that, in a number of bacillary dysentery outbreaks involving water, the organism was not, or could not be, isolated. Over several years of studying irrigation water in Colorado, Wang *et al.* (1956) and Dunlop *et al.* (1952) were not successful in isolating shigellae, although salmonellae were frequently isolated. The survival of shigellae in water appears to be shorter than that of many other bacteria; Dolivo-Dobrovolskiy and Rossovskaya (1956) found *Shigella* survival times of only 0.5-4 h during the warmest time of the year. However, enteric pathogens may survive much longer times in lake or river sediment than in free waters, and resuspension of such pathogen-loaded sediments at a later time may introduce a "slug" of bacteria into the waters which is not completely removed by treatment systems.

Estimation of Disease Potential by Direct Quantitation of Bacterial Pathogens

The detection of bacterial pathogens in water polluted with human or animal fecal matter is relatively easy when large numbers of organisms are present (American Public Health Association, 1975). Pathogenic bacteria have been isolated from relatively clean reservoirs, rivers, streams, and ground water; large samples, concentration techniques, and often elaborate laboratory procedures are used. However, detecting the presence of these pathogenic organisms in processed and disinfected water is far more difficult.

Scientific literature presents a vast array of media and methods for direct pathogen detection in finished water (reviewed by Geldreich, 1975, pp. 135-158). The greatest emphasis has been on the *Salmonella-Shigella* group of enteric organisms. Numerous modifications of well-known media are used for pre-enrichment, enrichment, selective inhibition, and isolation, and there are many recommended modifications of incubation temperature and time. Some methods use the classic most-probable-number (MPN) procedure for quantification; others use membrane filtration. Reviews of proposed procedures may be found in the *Journal of the Water Pollution Control Federation* (Geldreich, 1968, 1969, 1970b; Van Donsel, 1971; Reasoner, 1972, 1973, 1974, 1975). A recent review appeared in the fourteenth edition of *Standard Methods* (American Public Health Association, 1975).

There are serious limitations to the use of direct isolation of specific pathogenic bacteria for evaluating water quality. First, there is no single procedure that can be used to isolate and identify all these microorganisms. Second, only for salmonellae are the available procedures sufficiently accurate; the methods for other major pathogens--such as *Shigella*, *Vibrio*, and *Leptospira*--are inadequate. Third, none of the available procedures is applicable to quantitative isolation of small numbers of pathogens in drinking water. Fourth, even if procedures could be recommended, it is doubtful whether laboratories doing *routine* bacteriologic studies of water would have the expertise to carry out the procedures reliably.

In outbreaks caused by gross contamination, the standard procedures would be of value. Recently, Reasoner and Geldreich (1974) reviewed several of the rapid-detection methods proposed for water, and concluded that the cost per test, although perhaps higher than for conventional procedures, must be tolerated for potable-water quality assessment in emergency situations created by natural disasters, treatment breakdown, or rupture in the distribution network. None of these procedures would provide protection to the public as great as that provided by the currently-used indicator organism, the coliform.

Indicator Organisms

The term "indicator organism," as used in water microbiology, means a microorganism whose presence is evidence that pollution (associated with fecal contamination from man or other warm-blooded animals) has occurred. Indicator organisms may be accompanied by pathogens, but do not necessarily cause disease themselves.

As noted above, pathogens are usually more difficult to grow, isolate, and identify than indicator organisms, and often require special media and procedures. Indicator organisms, rather than the actual pathogens, are used to assess water quality because their detection is more reliable and less time-consuming. Pathogens appear in smaller numbers than indicator organisms and are therefore less likely to be isolated. An indicator organism should have the following characteristics:

- Applicable to all types of water.
- Present in sewage and polluted waters when pathogens are present.
- Number is correlated with the amount of pollution.
- Present in greater numbers than pathogens.
- No aftergrowth in water.
- Greater survival time than pathogens.
- Absent from unpolluted waters.
- Easily detected by simple laboratory tests in the shortest time consistent with accurate results.
- Has constant characteristics.
- Harmless to man and animal.

No organism or group of organisms meets all these criteria, but the "coliform group" of organisms fulfills most of them.

Escherichia coli and the Coliform Group

Escherichia coli is commonly found in the human intestine. It is not normally a pathogen, although pathogenic strains are known. Physiologically, *E. coli* and members of the genera *Salmonella* and *Shigella* are quite similar. All are classified as enteric bacteria of the family Enterobacteriaceae (Cowan, 1974). They are facultatively anaerobic, and are able to ferment sugars with the production of organic acid and gas. These three genera carry out a type of fermentation called "mixed-acid fermentation," but differ in a number of physiological characteristics. Many physiological differences between various enteric bacteria are known (Ewing and Martin, 1974), but at the beginning of the twentieth century this was not so. In the early days of water bacteriology, some simple operational distinctions were necessary. The lactose-fermentation test became the prime diagnostic tool: *E. coli* ferments lactose with the formation of acid and gas; *Salmonella* and *Shigella* do not ferment lactose.

One source of confusion is the necessity to distinguish between *E. coli* and the "coliform group" of bacteria. Although the taxonomy of bacteria is constantly undergoing revision, (see Buchanan and Gibbons, 1974, for the latest version) the genus *Escherichia* is well defined. It is distinguished from other mixed-acid fermenters of the Enterobacteriaceae primarily on the basis of sugar-fermentation reactions, motility, production of indole from tryptophan, lack of urease, inability to utilize citrate as sole carbon source, and inhibition of growth by potassium cyanide. However, the "coliform group" is not so precisely defined. The "coliform group," as defined in *Standard Methods* (American Public Health Association, 1975), comprises all "aerobic and facultative anaerobic, gram negative, non-spore-forming, rod-shaped bacteria which ferment lactose with gas formation within 48 hr at 35 C." This is not a taxonomic grouping, but an operational one that is useful in water-supply and sewage-treatment practice. It includes organisms in addition to *E. coli*, most importantly *Klebsiella pneumoniae* and *Enterobacter aerogenes*, which are not mixed-acid fermenters. The entry of the term "coliform" into sanitary bacteriology was associated with a policy established by H. E. Jordan when he became editor of the *Journal of the American Water Works Association*; he stated that he would substitute "coliform" for "*E. coli*" in papers submitted to him (Jordan, 1937).

Although most isolates classifiable as *Escherichia* by modern methods ferment lactose, about 5-9% of them do not (Ewing and Martin, 1974). No isolates of the genus *Salmonella*, either in the species *S. typhi* or in other species, produce gas from lactose (Ewing and Martin, 1974); therefore, a water sample containing *Salmonella* and a lactose-negative *E. coli* would be negative on the coliform test and would probably be discarded without further examination, because of the definition of "coliform." Even if glucose were substituted for lactose in a coliform analysis, a significant fraction of organisms would be missed, inasmuch as about 9% of isolates of *Escherichia* do not form gas from glucose (Ewing and Martin, 1974).

Because there are two procedures--the multiple-tube-dilution or most-probable-number (MPN) technique, and the membrane-filter (MF) technique--the coliform group of organisms requires two definitions (American Public Health Association, 1975). On the basis of the MPN technique, the group consists of all aerobic and facultatively anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with formation of gas within 48 h at 35C. On the basis of the MF technique, the group consists of all organisms that produce a dark colony (generally purplish-green) with a metallic sheen within 24 h of incubation on the appropriate culture medium; the sheen may cover the entire colony or appear only in a central area or on the periphery. These two groups are not necessarily the same, but they have the same sanitary significance.

If the coliform group is to be used as an indicator of fecal pollution of water, it is important to know that the coliforms do not lose viability in the water environment faster than pathogenic bacteria, such as salmonellae and shigellae. Little information exists on the survival of bacteria in finished water, and the data on other types of water are scattered and fragmentary. McFeters *et al.* (1974) recently reviewed previous work and presented their own data on die-off of intestinal pathogens in well water. As seen in Table III-3, die-off rates for pathogens and coliforms are approximately the same. Earlier work on the survival of salmonellae in water was reviewed by McKee and Wolf (1963).

TABLE III-3

Comparative Die-Off Rates (Half-Time)* Of Fecal Indicator Bacteria And Enteric Pathogens

Bacteria	Half-time (h)	Number of strains
Indicator Bacteria		
Coliform (avg.)	17.0-17.5	29
Enterococci (avg.)	22.0	20
Streptococci (from sewage)	19.5	
S. equinus	10.0	1
S. bovis	4.3	1
Pathogenic bacteria		
Shigella dysenteriae	22.4	1
S. sonnei	24.5	1
S. flexner	26.8	1
S. enteritidis, paratyphi A&D	16.0-19.2	2
S. enteritidis, typhimurium	16.0	1
S. typhi	6.0	2
V. cholerae	7.2	3
S. enteritidis, paratyphi B	2.4	1

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* Time required for 50% reduction in the population. Temperature, 9-12.5ºC. From McFeters et al. (1974)

Another factor to be considered is the relative sensitivity of coliforms and bacterial pathogens to disinfection. Although this subject has been studied little recently, the older work (Butterfield *et al.*, 1943; Butterfield and Wattie, 1946; Wattie and Chambers, 1943) indicated that there was essentially no difference between these different organisms in sensitivity to disinfection. This is not true when the coliform group is compared with viral pathogens. Viruses survive longer than bacterial pathogens (Colwell and Hetrick 1976). Gameson, A.L.H. 1974).

Some Deficiences of Coliforms as Indicator Organisms.

Coliforms meet many of the criteria for an ideal indicator organism previously listed; however, there are some deficiencies. There is aftergrowth in water--some strains do not disappear, but adapt to the new environment, and may become part of the natural flora. They are often found in waters, having entered through storm runoff. And they do not have constant characteristics; it is this property more than any other that has recently caused some water bacteriologists to question the continued use of coliforms as indicator organisms. False-negative and false-positive test results are not uncommon. The following summarizes the objections which have been raised by workers to the continued use of coliforms:

- Atypical lactose reactions, the concern of bacteriologists as early as 1899 (Parr, 1939), have occurred.
- Coliforms may be suppressed by high populations of other organisms, especially in untreated ground water (Allen and Geldreich, 1975) or where there is no free residual chlorine (Geldreich *et al.*, 1972).
- Coliforms do not represent a homogeneous group; it has been suggested (Dutka, 1973) that the definition of "coliform" include any organism defined by Edwards and Ewing (1972) as Enterobacteriaceae (this might require the addition of the oxidase test to the definition of the group, because all Enterobacteriaceae are oxidase-negative).
- The genus Aeromonas is a common cause of false-positive results in warm weather (Bell and Vanderpost, 1971; Ewing *et al.*, 1961; Ptak *et al.*, 1973). These can be eliminated by the use of the oxidase test, since Aeromonas is oxidase-positive and coliforms are oxidase-negative.
- False-negative results by strains that are unable to ferment lactose can give an unwarranted sense of security.

In the final analysis, testing for coliform, while not perfect bacteriologically, is still the most reliable indicator of the possible presence of fecal contamination and therefore of the pathogens that may be present in water.

Other Indicator Organisms.

Because of certain limitations of the coliform group as general indicators of water quality, workers have continually searched for better indicator organisms. No other organism has been found that is better than the coliform group, but it is pertinent to mention briefly some of these other indicators (Geldreich, 1975). *Fecal coliforms* (defined as those organisms which develop on media incubated at 44.5C), have frequently been used in stream and lake pollution work, but are not suitable for drinking water because the number of fecal coliforms is considerably lower in source waters than total coliforms, making the test less sensitive. *Fecal streptococci* (defined as those organisms able to grow on medium containing sodium azide) have been used in water pollution work, but have not proven suitable for drinking water analysis because of low recovery rates, poor agreements between various methods, and uncertainty as to their significance in water. Several other organisms have been suggested as indicators but have found even less acceptance: *Clostridium perfringens, Bifidobacterium, Pseudomonas, Staphylococcus*. It would be undesirable and extremely risky to substitute any organism for the coliform group now, although research studies that compare other indicator organisms with coliforms are warranted.

Rapid Methods for Coliform Counts.

There is great need for a rapid method of coliform counts which could give results in a shorter time than the 18-24 hours required by the membrane--filter method. A rapid method would permit early detection of system problems, and would considerably increase public health protection. Correlation of any rapid method used with accepted standards methods is essential, at least to the degree that the MF and MPN methods correlate. Unfortunately, no suitable rapid method for drinking water exists at present, but we will mention several methods briefly, in order to encourage research in this area.

One theoretically--feasible method would be a direct microscopic method, in which coliform cells would be concentrated on a membrane filter and stained by a specific staining procedure. The most commonly-suggested staining procedure has been the use of fluorescent antibodies specific for the coliform organisms (Danielsson, 1965; Ginsburg *et al.*, 1972). Unfortunately, this method is unlikely to succeed because there are at least 145 serotypes of *E. coli*, plus several more for the *Klebsiella* and *Enterobacter* group, so that a polyvalent antibody containing all of these antibodies would be necessary. Also, the number of cells in drinking water is likely to be so low that inconveniently long observation times would be necessary for quantifying the organisms present. Another disadvantage is that fluorescent antibodies might not distinguish living cells from dead, so that nonviable cells (perhaps resulting from a disinfection treatment) would also be counted.

Another rapid method that was suggested involves the use of radioactively-labeled lactose and measurement of the radioactive CO_2, liberated as a result of metabolism of the coliforms. This procedure was first suggested by Levin *et al.* (1961) and has been studied further by Scott *et al.* (1964). The EPA in Cincinnati (Reasoner and Geldreich, 1974) is currently attempting to improve this method. One of the desirable features of such a method is that it could be automated and run on-line. A disadvantage of the system is that, at present, it is not sensitive enough to detect ten or fewer organisms after 6 hours of incubation. It seems unlikely that such a method could ever be made sensitive enough to detect single coliform cells in 100-ml water samples, so that it probably will never replace standard methods, but it might prove useful in process control in large water systems.

The EPA at Cincinnati has also developed a 7-hour fecal coliform test, employing a membrane filter technique (Reasoner and Geldreich, 1974). Several other rapid methods which have been under study can be found in: Cady and Dufour (1974); DeBlanc Et Al., (1971); and Newman and O'Brien (1975).

Sampling for the Coliform Test

Water bacteriologists and sanitary engineers agree that the weakest link in the chain of water-monitoring and -testing is the collection of the sample. Too often this is left to the semiskilled or untrained worker.

The USEPA Interim Regulations (Federal Register, Dec. 24, 1975) follow the USPHS 1962 Drinking Water Standards; the number of required samples per month is based on the population served. Prior to 1925, when water quality standards were under the jurisdiction of the Treasury Department, bacterial sampling was left to the discretion of the local health and water utility officials, and varied widely according to local practices and the capacity of the laboratory. This, of course, was unsatisfactory, and was so recognized by responsible water utility people.

In 1941, a conference was called by the USPHS to revise the drinking water quality standards, and among the subjects under consideration were the frequency of sampling, the location of the sampling points, and the increased number of samples from the distribution system rather than from the plant final effluent. The deliberations of this 1941 conference, which resulted in the adoption of new standards in 1942, can be found in the following issues of the *Journal of the American Water Works Association*: 1941, 33: 1804; 1942, 35: 135; 1942, 35: 93; and 1941, 35: 2215-2226.

The principal changes adopted by the U.S. Public Health Service in 1941 were as follows:

1. The number of samples to be examined monthly from the distribution system would depend on the population served. During this discussion, the quality of the source water, the treatment procedure, the sanitary condition of the distribution lines, and the daily volume delivered to the consumers were also considered. Apparently, these latter factors made the final decision so complicated that in the final standard, only the size of the population served was used to decide on sampling frequency.

2. The detection of potential hazards in the distribution system due to faulty plumbing, cross-connection, post-contamination, and faulty plant and distribution system operation was included in the standards.

Samples were to be taken from representative points throughout the distribution system, with the frequency to be such as to determine the bacteriological quality of the water. The minimum number of samples per month was to be determined from the graph appearing in the Journal of the American Water Works Association (35:93-104, 1942). Apparently, this graph became the basis of the 1962 standards and the present interim regulations. According to F. Donald Maddox, Chief--Water Supply Systems Section, Region V, USEPA (personal communication to Walter Ginsburg), the subject of sample frequency was again discussed by the Committee that revised the 1962 Drinking Water Standards, and no changes were made. It is Maddox's opinion that the original 1942 curve was based on a number of water supply systems of different sizes which were known to have good treatment facilities and to be sampling at what was considered to be adequate frequency. The curve was plotted using these selected cities as a base line.

Richard L. Woodward, who served on the 1946 and 1962 Standards revision Committees, (personal communication to Walter Ginsburg) bears out the contention that the decision as to the actual number of samples to be examined was based on these elements of expert judgement.

One inherent weakness of this frequency graph has been discussed in the Federal Register (Dec. 24, 1975, p. 59566). It concerns the question of preparing monthly coliform averages from monthly percentages of positive samples. When four or fewer samples are examined each month, and one sample exceeds 4/100 ml, there is no way that the monthly average can conform to the recommended standards, even though the other three samples are negative. The regulations give the States the authority to average over a three-month period when four or fewer samples are required per month but this hardly affords a community the protection it needs when the water system is doing a poor job of treatment.

The Community Water Supply Survey by the USEPA in 1969 (McCabe et al., 1970) showed that 85% of the systems surveyed did not collect their minimum number of samples. Such inadequate sampling frequency can cause problems in the distribution systems to go undetected. (Geldreich, 1971; Miller, 1975).

In light of this discussion, it is clear that considerable research is necessary, with modern statistical methods, to develop better sampling protocols for water systems that serve different populations.

Coliform Standards

United States Standards.

The first U.S. national standards for bacteriological water quality were established in 1914 (Public Health Reports, 1914). These standards were specifically applicable to water used on interstate carriers, but were adopted quite early (formally or informally) by many states. Morse and Wolman, 1918, concluded that the standards are not precise and accurate indices of quality, but simply a convenient mode of analysis for comparative purposes which must be used with considerable caution.

It is obvious that with drinking water that meets the standards, there is no absolute assurance of the absence of pathogens, only confidence that their presence is unlikely; hence, the probability of waterborne-disease transmission is decreased. The decline in morbidity and mortality from some diseases, such as cholera, typhoid fever, salmonellosis, and shigellosis, provides some evidence of the validity of this confidence, although some of the decline may be due to the generally better health of the population, making people less susceptible to infection.

The latest standards adopted by the PHS were those of 1962 (U.S.H.E.W., 1962). Although none of the bacteriological numerical values was changed, a major procedural revision was made; the membrane-filter technique was accepted as an equivalent alternative to the multiple-tube-dilution (MPN) technique that had been in use since 1914. The MF standard was set at one coliform/100 ml.

The interim regulations of the EPA (Federal Register Dec. 24, 1975) have broadened the applicability of the standards to all public water systems, specified a sample size of 100 ml when the MF technique is used, and modified the required frequency of sampling. A monthly mean of less than one coliform/100 ml is still the standard.

International Standards.

Although many countries have their own drinking-water standards, two standards have international status--the World Health Organization European Standards (World Health Organization, 1970) and the International Standards (World Health Organization, 1971). In their preparation, individual national standards were considered.

The International Standards are proposed as "minimal standards which are considered to be within the reach of all countries throughout the world at the present time" (World Health Organization, 1970). They distinguish between piped supplies (roughly equivalent to the EPA definition of community public systems) and individual or small community supplies (comparable with the EPA definition of noncommunity systems) and between water leaving the treatment plant and that in the distribution system. For treated, disinfected supplies, water entering the distribution system should be of such quality that no coliform bacteria can be demonstrated (by the specified procedures) in 100 ml of water. For undisinfected water, the samples should yield no E. coli and three or fewer coliforms/100 ml. Within the distribution system, the standards are specified that "(1) Throughout any year, 95% of samples should not contain E. coli in 100 ml; (2) No sample should contain more than 10 coliform organisms per 100 ml; and, (3) Coliform organisms should not be detectable in 100 ml of any two consecutive samples." In nonpiped systems, the coliform count should not exceed 10/100 ml.

The European Standards are comparable to the International standards, but do not distinguish--in terms of quality--between disinfected and undisinfected water. The standard of 95% of 100-ml samples--showing no coliform bacteria throughout a year--"corresponds to an average density of about one coliform organism in 2 liters of water." Despite the numerical differences between the U.S. and the international standards, it is the intent of both sets of standards that coliform bacteria be absent from drinking water, to provide protection against disease.

Statistical Limits.

In discussing numerical standards of bacteriological quality of drinking water, the accuracy and the statistical limits of the tests must be considered. In using the classical, multiple-tube-fermentation test for coliform bacteria, it has been recognized that the procedure itself has a large inherent error (Morse and Wolman, 1918). A more recent discussion of these limits (Prescott et al., 1946) concluded that "because of the marked inaccuracy of the dilution MPN method ... any tendency toward fictitious accuracy in expressing the result should be discouraged." Table III-4, reproduced from Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1975), clearly shows this.

TABLE III-8
MPN Index And 95% Confidence Limits For Various Combinations Of Positive And Negative Results, Using Five 10-Ml Samples

No. of Tubes Giving Positive Reaction out of 5 of 10 ml Each	MPN Index Per 100 ml	95% Confidence Limits	
		Lower	Upper
0	<2.2	0	6.0
1	2.2	0.1	12.6
2	5.1	0.5	19.2
3	9.2	1.6	29.4
4	16.	3.3	52.9
5	>16.	8.0	Infinite

From American Public Health Association (1975).

The membrane filter technique, because it is equivalent statistically to a plate count, has a much smaller error. Thomas and Woodward (1955) compared the two methods and concluded "that, on the average, the former MPN gave higher indications of density by a factor of 1.0-1.9 with an average of 1.3 for the specific technics used ... However, the difference is not regarded as important from a practical viewpoint because of the inherent lack of precision of the individual MPN value." --and that, "With nearly all of the samples listed, the precision attained with a single filter was found to be two to five times greater than that of a 5-5-5-tube MPN" (note that the drinking water standards require only 5 tubes at one dilution with a corresponding greater lack of precision).

The Health Significance of the Coliform Test

Several pathogens, notably those in the genus *Shigella*, are able to initiate infection in humans even when introduced in very low numbers. Because it is not feasible to assay for bacterial pathogens directly in water, it is important to consider the utility of the coliform test in ensuring the bacteriologic safety of drinking water.

A direct approach to assessing the significance of the coliform count would be to obtain evidence of a correlation between numbers of coliforms and numbers of pathogenic bacteria (e.g., salmonellae or shigellae). One attempt to seek such a correlation was the study of Kehr and Butterfield (1943). Although imperfect, this is the only study found that attempts to relate the coliform count directly to disease incidence. In the discussion below, this study is analyzed in some detail in order to present a picture of the approach necessary to place the coliform standard on a firmer scientific basis. (It is perhaps of historical interest that the motivation for their study was an adverse decision by the Illinois Supreme Court as to the value of the coliform count in indicating that water is unsafe.)

The approach used by Kehr and Butterfield involved two aspects. First, data on the relative proportions of *Salmonella typhosa* and coliforms in various types of water (such as river, sewage, and sludge) were obtained from the literature, and the numbers of *S. typhosa* per 10^6 coliforms were calculated. These data, obtained from cities throughout the world, were then plotted on the ordinate on log-log paper, with typhoid fever morbidity on the abscissa (Fig. III-2). An approximately straight line was obtained. The point here was that the excretion rate of coliforms would be the same in a healthy as in a sick population, but that the latter would also excrete typhoid bacteria. Kehr and Butterfield then considered the stability of the *B. coli-S. typhosa* ratio and showed that *S. typhosa* and coliforms died off at approximately the same rate during sewage treatment, during self-purification in streams and lakes, and during drinking-water purification. The stability of this ratio is not surprising, when it is considered that *S. typhosa* and coliforms are members of the same group of bacteria and are likely to show similar tolerance and sensitivities to environmental influences.

From the data in Figure III-2 and from recorded waterborne outbreaks of typhoid fever, Kehr and Butterfield estimated a minimal infecting dose of *S. typhosa* for the general population and the percentage of persons infected by that dose. In doing this, these workers considered only epidemics of typhoid fever of a diffuse nature, i.e., characterized by a low attack rate but spread over a fairly large population. Kehr and Butterfield wrote that such epidemics were common at the time. Outbreaks with high attack rates, in which infection could arise more or less directly from carrier or patient discharge, were not considered, in order to avoid situations not likely to involve drinking water. In the diffuse typhoid epidemics, a common observation is the additional widespread occurrence of non-typhoid gastrointestinal disturbances. Considering the frequency of occurrence of the diffuse pattern of epidemics, and the data on concentrations of *S. typhosa* found in sewage and polluted waters, (as given in Figure III-2) Kehr and Butterfield concluded that it would be unlikely in such an outbreak for a person to drink more than a single typhoid bacterium or at most only a few, and that a single typhoid organism could produce infection in a small percentage of the general population. This conclusion is consistent with studies in experimental animals, which have shown that infection can be initiated by single bacterial cells (Meynell, 1961; Meynell and Meynell, 1965).

But this conclusion is not unequivocal. Populations of bacteria, even if derived from the same clone, can have a range of infectivities, and populations of humans have a range of susceptibilities. Thus, although a single cell may initiate an infection, not every cell-host contact will lead to infection. When the pathogenic bacterial population is diluted by a large volume of drinking water and spread over a large population of people, there is a non-zero probability that an appropriately virulent cell will reach a susceptible person. This is the situation that Kehr and Butterfield considered in their analysis of diffuse waterborne typhoid epidemics.

If the hypothesis that a single typhoid bacterium is infective can be accepted, then it is possible to consider the significance of the typhi:coliform ratio in drinking water. Assume that a water plant is treating source water with a typhi:coliform ratio of $10:10^8$, corresponding roughly to the ratio found in many polluted surface waters. Assuming equal destruction of typhoid bacteria and coliforms during treatment,

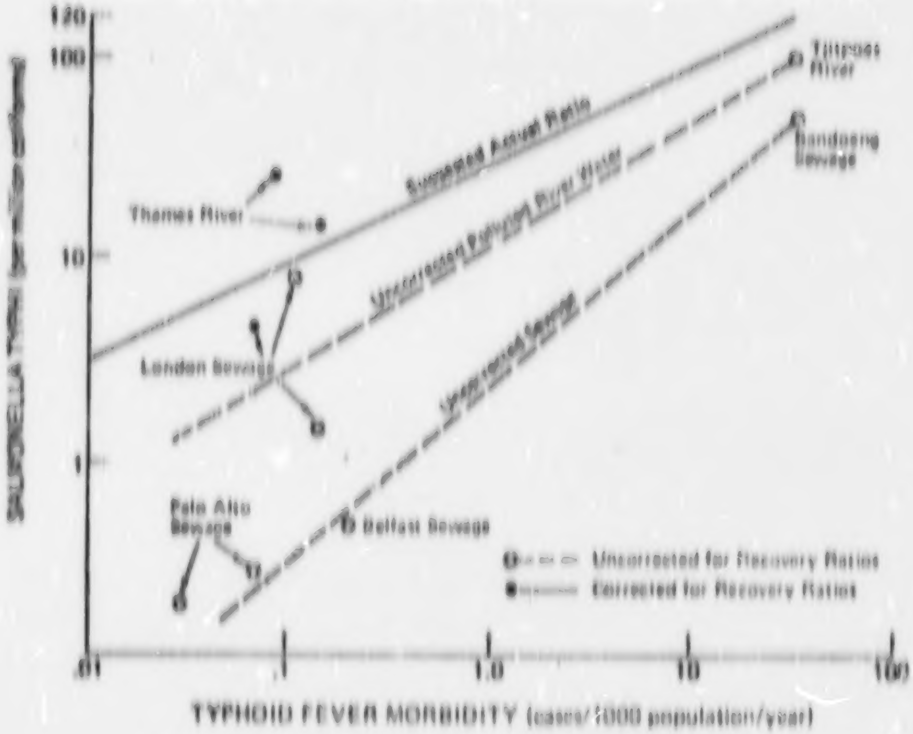

Figure III-2. Ratio of Salmonella typhi per million coliforms for varying typhoid fever morbidity rates. From: Kehr and Butterfield (1943).

if the finished water contained one coliform/100 ml (a reasonable possibility, even in many well-run plants), then the probability of consuming one typhoid bacterium when drinking a liter of water would be 10^{-5}; or, put another way, 10 people in a population of 1,000,000 could be infected. In an attempt to verify this, Kehr and Butterfield analyzed a number of epidemics of waterborne disease of the diffuse type, and concluded that the observed incidence of infection was consistent with this hypothesis. Thus, it was concluded that water which does not meet the coliform standard (one coliform/100 ml) can be responsible for waterborne disease, both gastroenteritis and typhoid fever.

Even more important, this analysis raises the question of whether water that meets the standard might bear disease-causing organisms. It is a simple exercise in arithmetic to convince oneself that this could be the case, inasmuch as drinking water with less than one coliform/100 ml, thus meeting the standards, could well have pathogenic organisms. Suppose that, instead of analyzing 100 ml of water, 1 liter were analyzed, and one coliform was found. This would be a coliform count of 0.1/100 ml, equivalent to one typhoid infection per 1,000,000 people (assuming the same typhi: coliform ratio as in the previous paragraph). Since the incidence of nonspecific gastroenteritis can be expected to be higher than that of typhoid, water that meets the present coliform standard may be the bearer of disease. However, existing epidemiologic data and reporting systems would not permit detection of such waterborne incidents, because the number of organisms would be below the detection limits of current surveillance methods. Although it is recognized that the above analysis is imperfect, it is the only one that has been done that this Committee is aware of.

The epidemiologic work of Stevenson (1953) added much weight to the rationale of establishing a coliform standard for drinking-water sources. His analyses showed that if raw water has fewer than 1,000 coliforms/100 ml, then it would be likely that salmonellae in finished water would be below infective levels.

Gallagher and Spino (1968) challenged the validity of the standards and stated that "summarized data from several stream surveys over the past few years showed little apparent correlation between quantities of total or fecal coliforms and the probable isolation of salmonellae." Geldreich (1970a) challenged their conclusion, on the basis of fecal coliform detections that showed a correlation between coliform numbers and salmonella isolations. He showed from numerous previous studies that, when fecal coliforms were 200/100 ml or more, there was a finite probability of isolating salmonellae. Smith and Twedt (1971) corroborated these data in a study of two Michigan rivers.

There are well-known epidemiologic histories of the presence of bacterial pathogens when the coliform index was low. Boring *et al.* (1971) reported that *Salmonella typhimurium* outnumbered coliforms by a factor of 10 in the Riverside, California outbreak. Similarly, a report by Seligmann and Reitler (1965) showed that index organisms can be low in the presence of pathogens. These sporadic reports of the failure of the index-organism concept emphasize the need for more research on pathogen detection.

Conclusion on Coliform Standard

Thus, we conclude that the current coliform standard is of value in protecting against frank outbreaks of bacterial diseases, but it may not protect against low levels of virulent pathogens. It is clear that further protection could be achieved by analysing larger samples for coliforms. More important than analysis of larger water samples for the protection of the public health--more frequent sampling, especially at more points throughout the distribution system--should be considered.

It has been reported repeatedly in the literature that the presence of any type of coliform organism in drinking water is undesirable. The regulations essentially demand that coliform-free water be distributed to consumers. Wolf (1972) has ably summarized: "The drinking water standard presently in use (approximately one coliform per 100 ml) is, in a sense, a standard of expedience. It does not entirely exclude the possibility of acquiring an intestinal infection. It is attainable by the economic development of available water supplies, their disinfection, and, if need be, treatment in purification works by economically feasible methods. It is not a standard of perfection."

It is not clear that the coliform standard provides comparable protection against virus disease. In fact, available information indicates that viruses may survive considerably longer than coliform bacteria outside the human host, and that the infectious dose may be very small (see section on viruses).

Good engineering and public health practices emphasize the need for using raw water of the highest possible quality, and for providing adequate sanitary-survey information. Bacteriological testing--or the imposed use of bacteriological standards--are an adjunct, not a replacement for good-quality raw water, proper water treatment, and integrity of the distribution system. The present coliform standards appear adequate to protect public health when: raw water is obtained from a protected source; is appropriately treated; and is distributed in a contamination-free system. Current coliform standards are not applicable for water reclaimed directly from wastewater.

The Standard Plate Count

The standard plate count (SPC) for drinking water, as described in *Standard Methods* (American Public Health Association, 1975), is the plating of small quantities (usually 1.0 or 0.1 ml) of a properly-collected water sample in a nutrient-agar medium (plate count tryptone glucose extract agar) and incubating aerobically for a fixed period at a prescribed temperature (35C for 24 or 20 C for 48 h).* The SPC is often referred to by other names, such as "total count," "viable count," "plate count," "bacterial count," "water plate count," and "total bacterial count." The organisms that develop into colonies on the agar plates represent a portion of the total population of bacteria in the water. This portion contains the bacteria that grow in the prescribed time in the specific environment provided. Although the method described is standard, there is no universal agreement on the acceptable concentration of organisms in drinking water. The most common allowable bacterial numbers used by health departments, water-supply agencies, and local jurisdictions vary from 100/ml to 500/ml of colony-forming units.

Geldreich (1973) has summarized several ways in which a standard based upon fecal-organism detection alone may not provide adequate health protection. Information gained from continued surveillance of water supplies by a standard plate count would provide an added degree of health protection. Even though water treatment is adequate and chlorine disinfection is provided, quality could deteriorate in the distribution system as a result of growth of organisms other than detectable coliforms. Finished water containing floc, or unfiltered turbid waters may carry organisms past the disinfection treatment, or the organisms may be protected by association with larger forms of life, such as nematodes (Chang *et al.*, 1960). Changes in water pressure in the distribution lines may cause release of organisms from protected areas, dead ends, or protecting materials in the system.

Geldreich *et al.* (1972) and Geldreich (1973) also reviewed the phenomenon of suppression of fecal organisms by the presence of large populations of organisms in common genera whose members normally show up in standard plate counts. These genera include *Pseudomonas*, *Bacillus*, *Streptomyces*, *Micrococcus*, *Flavobacterium*,

*In most tap waters, the number of organisms capable of growing at 20°C is considerably higher than the number growing at 35°. It is unlikely that most organisms growing at 20° will also grow at 35-37°C, and hence could not be pathogenic to man. A 20° count could still be of value in water works-practice, in providing information about filtration efficiency and about possible contamination of groundwater supplies.

Proteus, and various yeasts. It was concluded that the presence of 1,000 noncoliform organisms per ml could suppress the growth of coliforms. Geldreich (1973) speculated that this type of suppression of coliforms below detectable concentrations may have occurred in the Riverside, California, *Salmonella typhimurium* outbreaks, where coliform analyses did not reveal the contamination (Boring *et al.*, 1971). Plate counts over 500/ml also seemed to make detection of salmonellae and shigellae very difficult in a bacteriologic study of potable water in Karachi, Pakistan (Ahmed *et al.*, 1967). Although the genera of organisms detected by the SPC may not be harmful or dangerous to normal humans when present in drinking water in low numbers, under special circumstances (such as during therapy), these organisms are known to produce severe acute or chronic human infections (Geldreich *et al.*, 1972). Geldreich (1973) proposed that a 500/ml limit be placed on the standard plate count and that immediate investigation of water treatment and distribution systems be undertaken whenever the limits were exceeded. He recommended that water supplies be monitored routinely--at least every 3 months--to maintain the baseline data on the general bacterial population. A summary from Geldreich's (1973) paper follows:

> It should be clearly understood that the standard plate count is not a substitute for total coliform measurements of the sanitary quality of potable water. Rather, the use of a standard plate count limitation will:
>
> 1. provide a method of monitoring for changes in the bacteriological quality of finished water in storage reservoirs and distribution systems;
>
> 2. indirectly limit the occurrence and magnitude of *Pseudomonas*, *Flavobacterium* and other secondary pathogenic invaders that could pose a health risk in the hospital environment;
>
> 3. reduce problems in the detection of low densities of total coliforms due to interference by non-coliform bacteria;
> 4. monitor the effectiveness of chlorine throughout the distribution network and provide a warning of filter effluent-quality deterioration and the occurrence of coliform breakthrough; and,
>
> 5. indicate the existence of sediment accumulation in the distribution network that provides a protective habitat for the general bacterial population.
>
> Finally, the non-coliform bacterial population can be controlled by removing sediment and slime deposits from the distribution network followed by continuous application of

chlorine in sufficient dosage to insure the maintenance of a free residual throughout the system.

It should be emphasized that a standard plate count of 500/ml is attainable by water systems. Control of the general bacterial population in a variety of public water-supply distribution systems was demonstrated by Geldreich (1973), with a chlorine residue of approximately 0.3 mg/liter. In 60% of 963 water systems, standard plate count densities of 10/ml or less were obtained.

Although it is difficult to document, it is probably true that among the reasons for the decline in use of the standard plate count for drinking water are--the cost of the test, the lack of trained people to perform the number of tests needed, the difficulty of implementing and monitoring the laboratories needed, the resistance that would occur among the small and isolated water-plant operators, and similar issues. Although these are valid criticisms, they do not speak to the basic question of whether a regular system of plate-count surveillance would provide a tool for assessing the health hazards of water.

CONCLUSIONS ON STANDARD PLATE COUNT

The standard plate count is a valuable procedure for evaluating the bacterial quality of drinking water. Ideally, standard plate counts should be performed on samples taken throughout systems. The SPC has major health significance for surface-water systems that do not use flocculation-sedimentation filtration and chlorination, and for those ground-water systems that do no chlorination. When it is used, the sampling frequency should be at least 10% of the frequency of the coliform analysis, except that at least one sample should be collected and analysed each month.

The scientific information available makes it reasonable to establish the upper limit of the SPC initially at 500/ml, as developed in 35 C, 48-h plate count procedure (using the procedures prescribed by Standard Methods (American Public Health Association, 1975.)

Recommendations for Research on Bacterial Contaminants

A research program is needed to increase the value of the relatively simple bacteriological tests in cont [illegible] the sanitary quality of drinking water. The program should include:

1. Epidemiological studies of water quality and health, with application of more sensitive methods for detecting pathogens in drinking water and better reporting of outbreaks of waterborne disease.

2. Development of membrane-filtration methods to allow testing of larger samples and to reduce interference by overgrowth and disinfectants.

3. Improvement of procedures for making total-plate-counts and study of the utility of such tests for assessing the health hazards of drinking water.

4. Research on more rapid and sensitive methods for detecting pathogens and the use of such methods for monitoring the quality of water.

VIRUSES

Viruses differ fundamentally from other microorganisms that may occur in water. They are transmitted as submicroscopic, inert particles that are unable to replicate or adapt to environmental conditions outside a living host. These particles, or virions, have the potential to produce infections, and sometimes disease, in people who ingest them with drinking water. A viral particle eventually loses its infectivity with the passage of time, and with exposure to the rigors of its environment.

The viruses important to human health that are most likely to be transmitted by drinking water are the enteric viruses. These are primarily parasites of a portion of the intestinal tract. The stomach and duodenum are seldom affected by viruses, partly because of unfavorable conditions. Acid and proteolytic secretions predominate in the stomach; these and bile empty into the duodenum. Only viruses that can withstand such conditions will remain infectious and thus able to implant further down the digestive tract.

The most important human enteric viruses are the enteroviruses (ie., acid-stable picornaviruses), reoviruses, parvoviruses, and adenoviruses (Fenner _et al._, 1974). The virions of all these groups are roughly spherical, acid-stable and lack envelopes. All are relatively stable in the environment outside the host organism. Enterovirus particles are small (20-30 nm) and contain single-stranded ribonucleic acid (RNA). Reovirus particles are medium-sized (70-80 nm) and contain double-stranded RNA. Parvovirus particles are small and contain single-stranded deoxyribonucleic acid (DNA). Adenovirus particles are medium-sized and contain double-stranded DNA. Hepatitis A (infectious hepatitis) is transmitted by particles that closely resemble those of the enteroviruses (Provost _et al._, 1975).

Unlike bacteria, the viruses are obligate intracellular parasites, and cannot replicate in a cell-free medium. Many viruses can be grown in cultured cells _in vitro_. Some transmitted to man by water, most notably the virus of hepatitis A, have not yet been cultivated _in vitro_.

Ordinarily, viruses are detected and enumerated on the basis of their infectivity in cell culture or experimental animals. Testing of viruses for infectivity was greatly advanced by the finding that animal cells grown _in vitro_ would support the replication of human viruses (Enders _et al._, 1949). Infectivity resides in the nucleic acid portion of the virus particle. When a suspension of infectious particles is inoculated into a culture of susceptible cells, the particles are engulfed by, or penetrate host cells, and the cells produce progeny virus. Death of the cells often results.

If diffusion of the progeny virus particles is restricted by gelling the medium in which the cells are maintained, cell death occurs in a localized area called a "plaque." Such a plaque can be initiated by one viral particle, so that plaque enumeration has become an important method to measure animal viruses (Dulbecco, 1952; Dulbecco and Vogt, 1954). However, a plaque is not always initiated by a single viral particle, and many viral suspensions contain more aggregates than single particles (Sharp *et al.*, 1975); so the plaque-forming unit (PFU) is not an absolute basis for determining the numbers of viral particles.

Knowledge of viruses was acquired much more rapidly after the advent of cell-culture techniques. Nevertheless, transmission of enteric viruses by water had already been surmised by the time cell cultures became available.

History of the Enteric Viruses

The first reported epidemic of poliomyelitis in the United States occurred in New England (Putnam and Taylor, 1893). Caverly's (1896) observation that many cases had occurred in the Otter Creek Valley in Vermont suggested that the disease might be waterborne.

The poliomyelitis virus had been thought to infect people by the nasopharyngeal route. Kling (1929) found more virus in patients' stools than in their throats, but his findings were not generally accepted. Later, Harmon (1937) reported isolating virus by inoculating monkeys with colonic washings of four patients whose nasopharynxes yielded no virus. It was evident that infantile paralysis was not a strictly neurotropic disease and that the causative agent was strongly associated with the human intestines.

The picture was complicated by the presence of yet-unidentified agents other than the polioviruses in stools. The criterion of neuronophagia, seen in the spinal cords of inoculated monkeys, did not reliably distinguish the polioviruses from these other agents. In 1948, Dalldorf and Sickles reported isolating viruses from the stools of children by inoculation into suckling mice. The original stool specimens came from Coxsackie, New York, but "coxsackieviruses" were soon being discovered in many other locations.

When cell cultures became available, tests of stool suspensions began to yield viruses that were neither polioviruses nor coxsackieviruses (Robbins et al., 1951). The isolations of many such viruses were not clearly associated with any disease; these agents were eventually designated "enteric cytopathic human orphan" (ECHO) viruses. The National Foundation for Infantile Paralysis convened a committee to identify and classify these new viruses (Committee on the ECHO Viruses, 1955). The echoviruses were later classified with the polioviruses and coxsackieviruses to form the enterovirus group, members of which are numbered serially (Committee on the Enteroviruses, 1957, 1962).

The reoviruses were originally designated as members of the ECHO group. In 1959, Sabin suggested the term "reovirus" to apply to three serologic types of viruses which had common properties that differed from those of the echoviruses. The reoviruses are common in wastewater; 80% of the viruses which were isolated from wastewater by England et al. (1967) were reoviruses. The reoviruses have been recovered from persons with a wide variety of illnesses, but in no case has their etiologic role been established unequivocally (Horsfall and Tamm, 1965).

The adenoviruses (Rowe *et al.*, 1953) have produced large outbreaks of acute respiratory disease in military populations, and were a serious problem during World War II (Klein, 1966). They produce sporadic infections in the general population but are not often associated with overt disease. They occur frequently in sewage and wastewater and might thus contaminate drinking water. Their significance to the health of the general public is uncertain.

Probably the most important viral disease sometimes transmitted by water is hepatitis A. The disease was first described by Lurman in 1885. Its most common route of transmission is fecal-oral, primarily by person-to-person contact. In addition to water and personal contact, the disease is sometimes transmitted by food. The largest epidemic of hepatitis known to have been transmitted by water occurred in 1956 in Delhi, India; it was estimated that more than 30,000 cases occurred after sewage contamination of drinking water (Anonymous, 1957; Melnick, 1957). The virus has never been cultivated in cell culture, so that all available evidence about the transmission of infectious hepatitis by polluted drinking water is epidemiologic. Mosley (1967) suggested that the incidence of waterborne infectious hepatitis is grossly underreported, but estimated that less than 1% of the cases of this disease result from transmission by water.

Viruses that are still unidentified may also be responsible for waterborne disease, since there are many episodes of waterborne gastroenteritis and diarrhea of unknown etiology. Taylor *et al.* (1972) listed gastroenteritis as the most common waterborne disease, on the basis of number of outbreaks during 1961-1970. It is not clear which of the enteric viruses, if any, produce this common illness.

Epidemiology

Knowledge that a virus is shed in feces and may persist for a short time in wastewater is not proof that it is transmitted by water. Clearly, not every virus that is present in feces is waterborne. Viruses transmitted by a fecal-oral cycle can always be transferred directly from person to person, but this requires close contact. Several of the enteric viruses are known to be transmitted by water, but only some of the time.

Eight outbreaks of poliomyelitis in Europe and North America were eventually attributed to transmission by water, but Mosley (1967) believed that only one of them was adequately documented. This occurred in "Huskerville," Nebraska, in 1952; at least 45 people were made ill after contamination of a municipal water system.

The viral disease most frequently reported to be transmitted by water is hepatitis A (infectious hepatitis). Viral hepatitis became a reportable disease in the United States in January 1952, but the two types of hepatitis (i.e., infectious and serum hepatitis) were not separately notifiable until 1966.

The viral etiology of infectious hepatitis and transmission of the virus through water were adduced by Neefe and Stokes in 1945. Published reports of 50 hepatitis A outbreaks, attributed to contaminated drinking water, were summarized by Mosley (1967), who found the evidence of transmission by water to be convincing in only 30 of the 50 outbreaks cited.

Craun *et al.* (1976) reported 13 outbreaks of waterborne hepatitis that affected 351 people during 1971-1974. Although hepatitis A has been implicated epidemiologically in some 66 waterborne outbreaks since 1946 (Table III-5), there is no evidence of its transmission through correctly operated conventional water-treatment systems, except where defects in the distribution system have been found to be the source of contamination (Craun, 1976). About 40,358 cases of hepatitis were reported in 1974 (Center for Disease Control, 1974), but only a small fraction of these cases was transmitted by water.

TABLE III-5
Waterborne Outbreaks of Hepatitis-A by Source of Contaminaton
1946-1974

Cause	Municipal Systems	Semi-Public and Individual Systems
Untreated surface water	1	10
Untreated ground water	4	26
Inadequate or interruption of disinfection	3	5
Contamination through distribution system	11	1
Insufficient data to classify	3	2
Total	22	44

Epidemiologists have techniques which permit a decision as to whether an outbreak is waterborne or food-borne. These techniques require adequate and thorough reporting of the outbreak and a follow-up of infected individuals, to determine opportunities for contact with the disease agent. Since most waterborne outbreaks involve breakdowns or deficiencies in water-treatment systems, they are usually signalled as waterborne by their localization in one city or district, and by evidence from coliform counts of unsafe water.

Hepatitis A infections also result from the consumption of contaminated shellfish. (Denis, 1974; Dismukes *et al.*, 1969; Dougherty and Altman, 1962; Gard, 1957; Mason and McLean, 1962; Roos, 1956; and Ruddy *et al.*, 1969). Much information involving shellfish has been derived from studies with enteric viruses other than the hepatitis agent. Clams (Koff and Becker, 1969; Koff *et al.*, 1967; Liu *et al.*, 1966b), oysters (DiGirolamo *et al.*, 1970; Hedstrom and Lycke, 1964; and Metcalf and Stiles, 1965), and mussels (Bellelli and Leogrande, 1967; Bendinelli and Ruschi, 1969; and Duff, 1967) have been implicated. In water polluted with human feces, the shellfish accumulate enteric viruses (Liu *et al.*, 1967; Metcalf and Stiles, 1965; and Mitchell *et al.*, 1966), including the hepatitis virus. Humans have become infected by eating improperly-cooked shellfish. (DiGirolamo *et al.*, 1970; Koff and Sear, 1967; Koff *et al.*, 1967; and Mason and McLean, 1962). The shellfish themselves do not become infected (Chang *et al.*, 1971); rather, the virus is confined largely to their digestive tracts (Liu *et al.*, 1966b; Metcalf and Stiles, 1968). If the shellfish are removed to water that is not polluted, they will eventually free themselves of the virus (Liu *et al.*, 1966a; Metcalf and Stiles, 1968; and Seraichekas *et al.*, 1968).

Neither the common cold nor gastroenteritis is a reportable disease; these are probably the two most common (in the order named) human illnesses. Gastroenteritis has been called "sewage poisoning," "summer flu," and "unspecified diarrhea." Symptoms include nausea, vomiting, and diarrhea. No single etiologic agent has been identified; comparable illnesses may be caused by *Shigella sonnei*, *Salmonella typhosa*, enteropathogenic *Escherichia coli*, and *Giardia lamblia*, as well as nonbacterial agents (Craun and McCabe, 1973). The "Norwalk agent" (Kapikian *et al.*, 1972) and other viruses may eventually take their places among the known etiologic agents of gastroenteritis, but some questions as to how viruses may cause gastroenteritis have not yet been answered (Blacklow *et al.*, 1972). Infants whose intestines are infected with oral poliomyelitis vaccine virus do not show the symptoms of gastroenteritis.

There have been two outbreaks of gastroenteritis in the United States (one of which led to hepatitis) in which enteric viruses were recovered from the drinking water. In Michigan, restaurant patrons who drank unchlorinated well water became ill within 30 h (Mack *et al.*, 1972). Records showed that the well water had been contaminated with coliform organisms several times during the previous 6 months. No coliforms were detected initially in a 50-ml sample of the water. After 9.46 liters (2.5 gal) were concentrated by ultracentrifugation, both *Escherichia coli* and poliovirus 2 were recovered from the sample. No salmonellae or shigellae were recovered. A second 9.46-liter sample was tested after chlorination, but neither *E. coli* nor poliovirus could be recovered. In Dade County, Florida, the same migrant labor camp that had a water-associated typhoid outbreak in February 1973 (Pfeiffer, 1973) was the site of an outbreak of hepatitis A in March, 1975 (Wellings *et al.*, 1976). Samples of the chlorinated water taken at the camp nursery, rectal swabs from children in the nursery, and water from the evaporation pond, all yielded echovirus. Poliovirus also was isolated from the evaporation-pond water.

The present status of hepatitis A has been summarized as follows by Craun *et al.* (1976):

> There were thirteen outbreaks of waterborne viral hepatitis, affecting 351 people during 1971-1974. Over the past several years, there has been considerable controversy regarding the existence of viruses in treated water supplies and the possible health consequences. Hepatitis A has been epidemiologically-implicated in 66 waterborne outbreaks since 1946, and the data can be examined to determine how these outbreaks occurred (Table III-5). Of the 22 outbreaks occurring in municipal systems, three resulted from either inadequate or interrupted disinfection, and five were related to the use of contaminated, untreated surface or ground water. Half (eleven) of the outbreaks in municipal systems, however, occurred as the result of contamination of the distribution system, primarily through cross-connections and backsiphonage. There is no evidence, however, that the hepatitis A virus has been transmitted through correctly-operated, conventional water-treatment systems, except where distribution defects have been found as the source of the contamination. For the semi-public and individual systems, the use of contaminated, untreated ground water was the important factor responsible for outbreaks of hepatitis-A.

The cited epidemiological and laboratory evidence presents some paradoxes. The virus of hepatitis A has been shown, epidemiologically, to be transmissible in water, but has not been isolated in the laboratory from water samples. Gastroenteritis is transmissible in water; however, at least some of the time the disease is not caused by virus. Clearly, more research is needed to resolve these differences.

Recovery and Identification of Viruses

Factors Influencing Recovery

More than 100 different serological types of human enteric viruses may appear in wastewater (Berg, 1973). The chance that one or more of these viruses will appear in a community source of potable water is steadily increasing, as demands on available water make reuse increasingly necessary (Berg *et al.*, 1976). According to an EPA study of 155 cities that use surface-water supplies, 1 of every 30 gal that enter water-treatment plants has already passed through the wastewater system of a community which is upstream (Culp *et al.*, 1973). With the increasing use of renovated water, there is a need for methods that can detect the enteric viruses that might occur in raw or finished water.

Detection methods based on virus infectivity are more sensitive than immunochemical procedures, because more virus is needed to produce a perceptible immunochemical reaction than would ordinarily be needed to infect a cell culture. The amount of virus present in polluted surface-waters may vary over a wide range. A calculated value of 36 plaque-forming units (PFU) per gal, based on virus-shedding by children under 15 years old, was derived from data analyzed by Clarke *et al.* (1964). A total of 330 PFU/gal was recovered from a stream receiving wastewater effluent (Grinstein *et al.*, 1970). The available data suggest that raw potable water sources, subject to pollution from wastewater discharges, may contain significant numbers of enteric viruses.

Conventional treatment of a water supply (coagulation, sedimentation, filtration, and disinfection) to produce finished water is expected to result in removal or inactivation of a minimum of 6 $\log_{10}$ of virus (Clarke, 1976). Barring gross contamination of finished water, only low numbers of viruses are likely to occur in properly-treated supplies. Such small amounts of virus are likely to be detected only by highly efficient methods.

The general quality of the water is important in virus recovery. Turbidity, organic matter, pH, salts, and heavy metals all influence virus recovery. Both organic and inorganic solids suspended in water can adsorb viruses (Schaub and Sagik, 1975). In the testing of large volumes of water containing suspended solids, virus adsorption to these solids complicates the recovery process. Sample clarification that results in removal of the solids may also result in removal of virus. Deliberate use of solids for recovery of virus may not always succeed, because of failure to desorb the virus (Berg, 1973).

Organic material in water may influence the virus-recovery process by interfering with the ability of the virus to adsorb to a collecting surface (Wallis and Melnick, 1967a). The interference results in loss of the virus through penetration of the collecting surface. Organic matter can also protect the virus. Entrapment of enteric virus in fecal clumps would call for special procedures to disrupt the clumps, and thus release the virus.

Acid pH and metal cations are known to enhance adsorption of the virus to clay particles (Carlson *et al.*, 1968) and to membrane-filter surfaces (Wallis and Melnick, 1967b). Desorption of virus is favored by a pH of 9-11 and by the presence of organic substances (Cliver, 1967).

Knowledge of the influence of these factors can be used to avoid loss of the virus during sample collection, or can be applied to procedures to promote the recovery of the virus from water.

In summary, detectability of waterborne viruses is limited by: the low concentrations in which the virus occurs; by the presence of interfering substances in the water; by adsorption or entrapment of the naturally-occurring virus; and by inadequacies in the laboratory host systems (cell lines, experimental animals) in which isolation must ultimately take place.

Cell-culture Systems for Detection.

The types and quantities of viruses that can be detected depend on which cell cultures are used and how virus activity is manifested (plaquing versus cytopathology). Primary-monkey-kidney, or human embryonic-kidney cell-cultures were originally recommended for maximal efficiency in detecting enteric viruses (Lee *et al.*, 1965). One established primate cell line (BGM) was more sensitive than primary primate cultures in detecting viruses in water (Dahling *et al.*, 1974). In another study, 31 echoviruses were recovered in human, diploid-embryonic lung-cells, but not in monkey-kidney cells (Zdrazilek, 1974). A cell line which may eventually reduce the need for suckling mice in detecting coxsackievirus A has been evaluated by Schmidt *et al.* (1975). Plaquing (agar overlay) and cytopathic (liquid overlay) methods in combination were recommended for isolation of as many enteric viruses as possible (Hatch and Marchetti, 1975). The enteric viruses and the host systems that are required to isolate them are shown in Table III-6.

The best available combination of laboratory host systems will not detect the virus of hepatitis A, and will miss other viruses. Cultures that are susceptible to a given virus may differ in sensitivity, and wild strains of a virus may fail to

TABLE III-7

[a]Representative Virus Recoveries Cited for Methods Used to Recover Enteric Viruses from Water

Recovery System	Water	Initial Volume (liters)	Final Volume (ml)	Test Virus	Initial Virus Concentration (PFU/)	Virus Recovery (per cent)	References
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

P 1, 2, or 3 - Poliovirus 1, 2, or 3
E7 - Echovirus 7
[illegible] - [illegible]
[illegible] - [illegible]
[illegible] - [illegible]
[illegible] - [illegible]

* - [illegible]
[illegible] - [illegible]

[a] [illegible]

form plaques (or to produce cytopathology in the same way) as laboratory strains.

Recovery Procedures for Virus in Finished Water.

There are extensive reviews of methods for recovering waterborne viruses (Hill et al., 1971; Sobsey, 1976). Present methods seem generally adequate for recovery of enteroviruses, but perhaps not of reoviruses and adenoviruses (Fields and Metcalf, 1975).

Representative recoveries of virus from seeded water-samples are shown in Table III-7. Although there is no universal method for concentrating all enteric viruses from water, some methods are quite effective for some of the enteric viruses in some types of water. Flowthrough-filter adsorption-elution systems and ultrafiltration methods with anisotropic, polymeric membranes in tangential-flow systems are the best for recovery of small quantities of enterovirus in large volumes of treated or finished water. Virus concentration from water by adsorption to precipitable salts or polyelectrolytes, aqueous polymer two-phase separation, reverse osmosis, hydroextraction, continuous-flow ultracentrifugation, and forced-flow electrophoresis methods can generally be applied only to samples of a few liters. Therefore, they are not suited to recovery of a low number of infectious units of virus from large samples of water.

Identificatin of Viruses Recovered from Water.

Serologic methods for virus identification are often based on virus infectivity. In the serum-neutralization test, infectivity is blocked by the action of a homologous antibody. Neutralization tests, using combination pools of enterovirus equine antisera, are one of the reliable ways of identifying an enterovirus isolate (Melnick et al., 1973). These tests are usually completed within 48-72 h in cell cultures, after recovery of the isolate and passage in cell culture. The total time required for completion of all these procedures depends on virus growth-rates and may vary from a few days to weeks.

Immunochemical methods have been evaluated as a more rapid means of virus identification. Immunofluorescence (Katzenelson, 1976) and immunoenzymatic (Kurstak and Morisset, 1974) procedures have been tested. These procedures are based on antigen-antibody interactions whose results can be seen sooner than the visible cytopathic effects required for interpretation of serum-neutralization tests.

The concept of an indicator bacterium was discussed in some detail earlier in this report. Indicator bacteria are organisms which, although not pathogenic in themselves, are constantly present in intestinal discharges, so that their detection in the environment constitutes a signal for possible fecal pollution. Is it possible to consider certain viruses as indicator viruses? Such indicator viruses might provide a clue to the inadequacy of source-waters, or water-treatment systems, in terms of virus contamination or removal, and hence could be useful in the control of waterborne virus disease.

If a rapid serologic-identification test were available, it might be adaptable to detection of some indicator virus whose presence has correlated with that of other enteric viruses. The polioviruses (which are common in wastewater and easy to detect) have been proposed as indicators of other enteric viruses that are more difficult or impossible to detect by laboratory methods. A rapid immunochemical method for detecting polioviruses might be attainable, but this does not validate the use of polioviruses as indicators. Although polioviruses are the most common group of enteric viruses recovered from polluted waters, they are not always predominant or even present among the enteric viruses in field samples (Grinstein *et al.*, 1970; Metcalf *et al.*, 1974b). Because recovery methods are essentially the same, whether for polioviruses or for all the enteric viruses, there is little to be gained by adopting such an indicator virus now.

Sensitivity of the Flowthrough Method.

Laboratory-based trials of flowthrough equipment and methods, using at least 100 gal of tapwater experimentally contaminated with poliovirus, regularly yielded positive results from 500-gal samples that contained 3-5 PFU/100 gal, and sometimes only 1-2 PFU/100 gal (Hill *et al.*, 1976). Members of the reovirus, echovirus, and coxsackievirus groups A and B, at inputs of 1 PFU/gal or less, were also recovered when 100 or 500 gal of water were sampled. Emphasis was placed on ability to recover small quantities of virus in large volumes of finished water. Performance at extremely low concentrations of virus was considered a better index of the merits of a method than high-percentage recoveries based on very large test doses. The success of the laboratory trials has led to inclusion of flowthrough procedures in the fourteenth edition of Standard Methods for the Examination of Water and Wastewater (1975) as a tentative method for finished water.

Perspectives in Testing Large-volume Samples.

Some of the assets of the flowthrough method may also be viewed as liabilities. The portable concentrator permits the testing of large-volume samples without transporting great quantities of water to the laboratory; however, a probable consequence is that the laboratory virologist is not answerable for the validity of the field-sampling procedures. The method enables detection of as little as 1 PFU of virus in a 100-gal water sample, but the authenticity and significance of a positive test-result is questionable when very few PFU are found. The method can detect only viruses for which susceptible tissue cultures or laboratory animals are available, so that the agents of hepatitis A and most viral gastroenteritides will be missed. The vaccine polioviruses have the highest overall probability of occurrence and detection, but they do not themselves constitute a threat to human health. The vaccine polioviruses, when compared to coliform organisms, may constitute a more sensitive or valid indicator of the threat of water-borne virus pathogens; however, this kind of comparison should be made only after more coliform tests have been performed on much larger samples than 100 ml. Both the flowthrough method of virus collection and larger-volume coliform tests may have a future as standard methods for discretionary, intensive testing of finished water; neither should be regarded as a routine monitoring method for on-line quality control.

Health Effects of Viruses in Drinking Water

Large numbers of enteric viruses are present in some human feces, and therefore in wastewater. These viruses may not be completely removed or inactivated by wastewater treatment, so they may be discharged to surface water that serves as another community's raw-water source (Clarke et al., 1964). If the viruses are not completely removed or inactivated by water treatment, they may be ingested. People infected by these ingested viruses do not always become ill, but disease is a possibility in persons infected with the agent of hepatitis A or any of the other enteric viruses. Several diseases involving the central nervous system, and more rarely the skin and heart, are caused by the better-characterized enteroviruses: polioviruses, coxsackieviruses, and echoviruses. Some reoviruses and parvoviruses have been implicated in nonbacterial gastroenteritides. More than 100 serotypes of enteric viruses are known and are recovered from wastewater from time to time (Davis et al., 1967). Sporadic occurrences of echoviruses and coxsackieviruses in a susceptible population may result from transmission of the viruses through water, but this has yet to be proven.

Poliovirus infection by the oral route has been studied to some extent, especially in connection with the development of attenuated poliomyelitis vaccines for oral administration. The remainder of the enteric viruses have been used only to a small extent in research of this kind. In the few studies of other orally-administered viruses, there was usually no attempt to determine how much virus was needed to produce infection, or even how much virus was being administered.

Some of the particles in any collection of virus are perceptibly defective. The rest apparently possess all the constituents necessary to initiate infection of a cell and, by successive replication, of a host organism; such a particle might be described as "nondefective."

A nondefective virus particle, inoculated into a culture of susceptible cells, may or may not cause an infection. Incipient infections may be aborted in one way or another, so the number of apparently nondefective particles greatly exceeds the number of tissue-culture infections they can produce. Schwerdt's (1957) ratios of particles to tissue-culture doses (the quantities of virus required to produce perceptible infections in a tissue-culture, whether the infections are perceived by cytopathic effects, plaque formation, or some other manifestation) were about 36:1 to over 100:1, whereas Joklik and Darnell (1961) reported a ratio of approximately 250:1. The latter study showed that the particles that had not succeeded in initiating infections were not defective; but the ways in which incipient infections were seen to abort would not account for an infectivity ratio as high as 250:1.

However, only one poliovirus particle ordinarily infects a cell. Thousands of nondefective, genetically identical progeny particles are produced in replication. Once a cell is infected, its neighbors in a culture are virtually certain to become involved. Nevertheless, if small numbers of nondefective particles have been inoculated at the outset, infection of a first cell is unlikely to occur.

Poliovirus infection by the oral route had to be studied during development of the "live-virus" poliomyelitis vaccines. Sabin found that nonhuman primates could not be substituted for man in such investigations, because of species differences in the relative susceptibility of the intestines to poliovirus infection. He reported that, if fewer than 10^5 tissue-culture doses of vaccine poliovirus were ingested by a human, the virus would bypass the pharynx, but would infect the intestines (1957).

Ratios of physical particles to oral, infectious doses for man, comparable with those for tissue-culture doses, have not been reported. Orally-administered poliovirus doses have therefore been expressed in terms of tissue-culture doses, even though infections of these two host systems have never been shown to be analogous. Indeed, the disagreement (Bodian, 1957; Sabin, 1957) over which intestinal cells are responsible for replication of poliovirus still seems to be unresolved. Horstmann (1961) described 10^5 tissue-culture doses as "an average minimal reliable dose for most strains," and thought it remarkable that some strains would infect by the oral route when fewer than 10^4 tissue-culture doses had been ingested.

The subjects in several of the early poliomyelitis-vaccine trials were infants in their first few days or months of life. They included babies born to mothers who had various serum concentrations of antibody against polioviruses. The criterion of infection was the shedding of the virus in the infant's feces after 1 week. Though large amounts of virus were often administered, not all vaccinated infants became infected. The findings of several investigators are summarized in Table III-8. A study by Koprowski (1956) showed that two of three children, each fed 2 PFU of vaccine poliovirus in enteric-coated capsules, became infected. Plotkin and Katz (1967) presented data that they believe demonstrated a parity between the quantity of vaccine poliovirus needed to infect a tissue-culture, and that which will infect a human subject by the oral route. Studies by others indicated a need for more than 10^4 tissue-culture doses of vaccine in infants (Gelfand _et al._, 1961).

TABLE III-8
Results of feeding various quantities of oral
poliomyelitis vaccine to infants

Dose	No. Infected/ No. Fed	Percent Infected	Reference
PFU*			
0.2	0/2	0	Koprowski, 1956
2	2/3	66	"
20	4/4	100	"
5.5×10^6	16/18	89	Holguin *et al.*, 1962
TCD50**			
$10^{3.5}$	28/97	29	Lepow *et al.*, 1962
$10^{4.5}$	52/96	56	"
$10^{5.5}$	58/85	57	"
$10^{6.6}$	12/20	60	Krugman *et al.*, 1961
$10^{7.6}$	15/20	75	"
$10^{5.5}$	206/300	68	Warren *et al.*, 1965
$10^{5.3}$	133/159	79	"
1	3/10	30	Katz and Plotkin, 1967
2.5	1/9	11	"
10	2/3	67	"
$10^{5.5}$	4/8	50	Gelfand *et al.*, 1960
$10^{7.5}$	9/9	100	"

*Plaque-forming units

**TCD50 = tissue culture dose 50%

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The available data on polioviruses have important limitations; unfortunately, far less information seems to be available concerning infectivity, by the oral route, of other intestinal viruses. The dose of coxsackievirus B5 that would infect 50% of newborn mice to which it was administered by the oral route was between 10^2 and 10^3 tissue-culture doses in studies by Loria *et al.* (1974). Their criterion of infection was disease, so they were measuring the pathogenic dose, rather than the infecting dose. No other reports of attempts to determine the minimal infecting dose of ingested human intestinal viruses seem to be available--whether for humans or other species--in terms of tissue-culture doses.

In sum, poliovirus infection at the cellular level appears to be initiated by a single nondefective particle. If the ratio of nondefective particles to infections is the same for ingested virus, this is fortuitous; the yet-to-be-identified cells in the intestines to which the particles must attach may be either more or less susceptible than tissue-culture cells, just as the susceptibility of different tissue-culture cells may vary. Counts of virus particles are not likely to be useful in this application, and some important intestinal viruses cannot be enumerated in tissue-cultures at all. There is no valid basis for establishing a no-effect concentration for viral contamination of finished drinking water.

Virus Removal in Water Treatment

When viral disease occurs in association with a drinking-water supply, one first has to consider the possibility that cross-contamination or back-siphonage may have admitted wastewater into the distribution system. However, this source will not be discussed here; the integrity of water-distribution systems is a technological, rather than a virological, problem. If the integrity of a system is not maintained, the most immediate consequences are more likely to involve enteric bacteria than viruses.

Alternatively, viruses may occur in a community's raw water supply as a result of intended or inadvertent "recycling" of wastewater. If viruses are not removed or inactivated (a virus is said to be inactivated when it loses its ability to produce infection) in the treatment of the water, disease may occur among those who drink the finished product. The required degree of virus-removal or inactivation cannot be stated explicitly, because of lack of good information as to how much virus must be ingested to cause disease. When goals in virus removal are considered, it is also important to remember that less than 1% of water consumed by a community is used internally by humans; more than 40 gal of water per person per day are used for household purposes (Witt *et al.*, 1975).

One would like to know what amounts of virus might occur in raw water at the point of intake. Most reports of virus in raw water are not quantitative: for example, Foliguet et al. (1966a) reported detecting enteroviruses in 13 of 162 raw-water samples tested in France during 1961-1963, but one cannot determine the volume of water that each sample represented. The highest virus concentrations measured at or near water intakes by Berg (1973) were somewhat less than 0.2 PFU/l of water. Available data (Chang 1968) indicate that 10 PFU/l constitutes a "reasonable virus load in moderately polluted water." One would hope to remove or inactivate all viruses before the treated drinking water were considered "finished."

Berg (1973) has offered to resolve this difficulty by requiring that reclaimed and other potable water be disinfected so as to destroy at least 12 logarithmic units of a reference virus at 5C and that the finished water be tested frequently to ensure that there is not more than 1 PFU in a 100-gal (379 liter) sample of the final product. An apparatus that will accommodate such samples has been developed. Recently, 4 of 12 samples (100-105 gal, or 379-397 liters, each) of finished water from a modern, well-operated plant serving 600,000 people in Fairfax County, Virginia were shown to contain 1-4 PFU of poliovirus each (T.G. Metcalf, unpublished data). The public-health significance of such findings is uncertain. The infectious dose of poliovirus by the oral route is not yet known, and the detected virus was apparently of vaccine origin and therefore nonpathogenic. Any virus detected in finished drinking water may indicate that other viruses, not detectable by laboratory techniques, are also present; however, no outbreak of human illness has been associated with the water supply that was examined.

The detection of very few tissue-culture doses of virus in extremely large samples does not yet support the adoption of a numerical specification for the volume of finished water from which detectable virus must be absent, or for the projected viricidal effectiveness of a disinfection process. Safety of drinking water, from the virus standpoint, cannot at present be defined in numerical terms.

None of the unit operations and processes used today in the treatment of water supplies were devised originally to remove or destroy viruses. Still, almost all these treatments have some antiviral effect. The potential antiviral effectiveness of disinfection (chlorination) probably exceeds that of all other available treatments combined. Any treatment for which there are substantial data to indicate ability to remove at least 50% of incident viruses will be considered here. Many of the data have been obtained from bench-scale studies with experimentally inoculated model viruses. Accurate measurement of virus removal, (by a treatment being evaluated) is possible only if the initial concentrations of viruses are very high. The required concentrations of viruses would not occur naturally in water destined for human consumption (except in reclaimed wastewater), and would be difficult to attain by adding laboratory viruses to pilot-scale batches of water. Such experiments are conducted under more closely-controlled conditions than may be attainable in practice, and the aggregation that is always present in virus suspensions may differ in degree between inoculated virus and naturally occurring virus. Results will not be reported here to a greater degree of precision than seems reasonable to expect in practice.

Coagulation and Settling.

An early step in the treatment of raw water often includes coagulating suspended solids and allowing them to settle to the bottom of a tank. The supernatant water is then collected for further treatment. Substances added to induce coagulation may include primary flocculants (such as aluminum sulfate and ferric chloride,), coagulant aids (polyelectrolytes), acids, alkalis, and even clay used for supplemental turbidity. Four recent, extensive studies indicated that virus removal from 90% to well above 99% can be achieved by coagulation and settling under carefully controlled conditions (Foliguet and Doncoeur, 1975; Manwaring *et al.*, 1971; Schaub and Sagik, 1975; and Thorup *et al.*, 1970). Either aluminum sulfate or ferric chloride proved a good primary flocculant for virus removal. Polycation-coagulant aids were useful in virus removal, whereas anionic or nonionic polyelectrolytes probably would not be. Clays are sometimes added, to ensure formation of a visible floc to which virus may adsorb. Organic matter in the water interfered with virus removal by coagulation and settling; this effect may be prevented by prechlorination or preozonation. Finally, it should be noted that the virus removed had not been inactivated. Virus adsorbed to floc was quite capable of causing infection.

Filtration and Sorption.

Virus particles are too small to be retained mechanically on sand, or on most alternate media used in water filtration. Virus retention by such media depends on association of the virus with suspended matter that is large enough to be trapped mechanically, or by direct sorption of free virus-particles to the surface of the filter medium.

When sand filtration follows coagulation and settling in the treatment scheme, viruses that sorbed onto fine floc particles can be retained efficiently by sand (Foliguet and Doncoeur, 1975). Virus removal by the filter ranges from 90% to more than 99%, with the very highest figures resulting when the virus was poorly removed by previous settling of the floc. Sand, by itself, is reputed to be a poor medium for removing viruses from water (Chang, 1968). However, Lefler and Kott (1974) removed more than 99% of their model viruses by slow sand filtration when the suspending water contained calcium chloride and magnesium chloride at 0.01 *M*.

Diatomaceous earth has been evaluated by Brown *et al.* (1974) as a filter medium for removing viruses from water. The medium was coated with ferric hydroxide or a synthetic polymer developed for water treatment. The model virus was removed from dechlorinated tapwater (pH 9.5) to the extent of approximately 98%, but the process was less effective at a lower pH (6.7-6.8), or in the absence of coating. It is not clear whether this filtration process was intended principally to follow or to supplant the coagulation and settling-steps in water treatment.

Activated carbon is another possible sorbant medium for removing viruses. Cliver (1971) found that retention of virus on the medium could be 90% initially, but was apparently lower after a column had been operated for some time. Relatively little desorption seemed to occur when water, rather than wastewater, was treated.

The possibility of virus desorption from a filter-medium, or of resuspension of floc to which virus has adsorbed, must always be considered in evaluating treatments such as those described above. None of these treatments appears to inactivate the virus that has been retained. Indeed, if any of these treatments proves less effective in practice than was indicated in bench-scale studies, it may well be because of desorption or resuspension, rather than inadequate initial retention of virus.

Water-softening.

Softening of water at the treatment plant may inactivate virus, rather than simply remove it. Sproul (1971) summarized research in his laboratory that showed modest virus removal with straight-lime softening, and more than 99% removal with sodium hydroxide precipitation of magnesium ions, with excess lime-soda ash softening. He used the term "inactivation" to describe this removal, but he compared data from his and other laboratories which indicated that viral infectivity is lost only in some cases at a pH near 11, which occurs in excess lime-soda ash softening.

Disinfection.

If all of the treatments described above were applied sequentially to raw water, one could expect a total of 6-12 logarithmic units of virus-removal or destruction, without recourse to chemical disinfection. However, chemical disinfection seems to be more reliable than the other treatments.

Most chemical disinfectants that are effective against viruses are strong oxidising agents. The general principles of action of these substances on viruses have been discussed by various authors, not all of whom reached the same conclusions. Liu *et al.* (1971) emphasised differences in resistance among the groups of enteric viruses (as well as the importance of clumping of virus particles) as a source of confusion in interpreting inactivation data. Given the contact-times likely to be used, these differences are probably not critical to the success of an efficient disinfectant. However, Kruse *et al.* (1971) emphasised that not all slowly-effective forms of disinfectants can be made to accomplish their task by extending contact time. Treatment prior to terminal disinfection accomplishes two purposes--reducing the virus load and preparing water for more effective terminal disinfection, by removal of interfering substances.

Chlorination.

Chlorine, applied in its elemental form or as hypochlorite, is the standard of disinfection against which others are compared. Depending on the pH of the water and on the presence of ammonia, the chlorine may take the form of HOCl, OCl^-, Cl_2, or chloramines. The viricidal effectiveness of these forms apparently decreases in the order listed.

Neefe *et al.* (1947) studied chlorine inactivation of the virus of infectious hepatitis (in drinking water which was given to human volunteers). The results were not quantitative, but they showed that, if viruses were added (as a suspension of infectious feces) to water which was then coagulated and filtered, a chlorine-dose sufficient to yield a free residue of 0.4 mg/l after 30 min of contact, would render the virus noninfectious for the volunteers. Nothing has been done to refute or augment these findings in the intervening period of almost 30 years.

Clarke and Kabler (1954) showed that coxsackievirus A2 was inactivated more slowly by chlorine at a pH of 9 than at a pH of 7, and more slowly at 3-6C than at 27-29C. Not surprisingly, the inactivation rate also depended on the concentration of free chlorine (hypochlorous acid and hypochlorite ion). Kelly and Sanderson (1958, 1960) extended these results to other human enteroviruses and showed that the antiviral action of chloramines is a great deal slower than that of free chlorine.

Clarke *et al.* (1956) found adenovirus 3 to be more susceptible to chlorine than the enteroviruses; destruction rate of the former was said to be comparable to that of *Escherichia coli*. Liu *et al.* (1971) showed that the reoviruses were even more chlorine-sensitive than the adenoviruses, and that some of the differences in chlorine sensitivity among enteroviruses might be related to the degree of aggregation of the viruses in suspension. The aggregation always seen in virus-suspensions imposes significant limits on the predictability of virus inactivation (Sharp *et al.*, 1975).

Hypochlorous acid, which predominates below a pH of 7.5, is more active against viruses than is hypochlorite ion (Chang, 1968). Where the water contains nitrogenous substances, chlorine should be added to the "breakpoint," beyond which any further added chlorine will be free to inactivate viruses (Kruse *et al.*, 1971). Chlorine dioxide (ClO_2) may offer some advantages over other forms of chlorine because it is less reactive with ammonia, and less affected by temperature and pH (Chang, 1968).

Bromination.

Bromine has been studied (to a limited extent) as an alternative to chlorine in inactivating waterborne virus (Taylor and Johnson, 1974). It is clearly effective, and it may be somewhat less subject than chlorine to "demand" losses in use. Bromamine, for instance, is capable of significant virus inactivation. More study will be required before bromine can be considered likely to supplant chlorine as a general disinfectant. It does have advantages for some specialized uses.

Iodination.

Iodine is also capable of inactivating enteroviruses in water under controlled conditions (Berg *et al.*, 1964). Although relatively high doses may be required, Chang (1968) suggested that iodine may be useful in special situations. Studies by Olivieri *et al.* (1975) on the comparative modes of action of the halogens on a small bacterial virus (f2) indicated that iodine reacts with the coat protein, whereas chlorine probably inactivates the viral nucleic acid.

Ozonation.

Ozone is finding increasing use as a water disinfectant and is proving effective against viruses. Although Majumdar *et al.* (1973) found that ozone was relatively ineffective against poliovirus when the disinfectant was present at less than 1 mg/liter, Katzenelson *et al.* (1974) found ozone to be faster and more effective than chlorine against poliovirus, even at concentrations as low as 0.3 mg/l. Ozone does seem to offer significant advantages as an antiviral disinfectant for water. Its most conspicuous liability is that its activity cannot be sustained in the water all the way to the consumer, so that it does not provide any protection against post-treatment contamination.

Effectiveness of Water Treatments.

Virus may occur in some raw water; standard treatments, such as coagulation and settling followed by rapid sand filtration (if carefully performed under practical conditions) could remove a combined total of 2-6 logarithmic units of virus present. Viruses removed in these ways are not inactivated.

A virus that is still present can be inactivated by chemical disinfection. Ozone and chlorine seem most clearly-suited to community use for water disinfection. With raw water that sometimes contained virus, both ozone and chlorine were found to produce finished water in which viruses could not be detected by the methods used (Foliguet *et al.*, 1966b). In general, it appears that current treatment technology, diligently applied, can consistently produce finished water in which viruses are not likely to pose a threat to public health (Clarke *et al.*, 1974).

The theoretical model for virus removal or inactivation by each treatment, assumes that a zero concentration of virus will never be attained. This implies that testing ever-larger samples of finished water will, in some instances, result in detection of a virus. If the presence of virus in finished water results from undertreatment of virus-contaminated raw water, treatment should be intensified (to include coagulation, settling, filtration, and chemical disinfection, such as breakpoint chlorination, if these are not already being done), and efforts should be made to alleviate the contamination of raw water.

Bacterial Indicators and Viruses in Drinking Water

It should be clear from the preceding discussion that virologic tests are not "routine" in the sense that many bacteriological methods are routine. Can bacteriological methods be of any value in ensuring the virological safety of drinking water?

Viruses differ fundamentally from bacteria in size and in biological properties. Decreases in concentrations of bacterial indicators during water treatment ought not to be expected to correlate directly with virus removal or inactivation. Competent execution of water-treatment methods ultimately determines the safety of the finished product.

In the event of a treatment-break or a loss of integrity of the distribution system, the presence of excessive turbidity, coliforms, or standard plate counts will indicate the existence of an unsafe situation and a possible viral hazard. Even though these indicators are only incidentally-correlated with the presence of virus in drinking water, they are important, because they will evoke remedial action. Detection of viruses themselves in drinking water will take at least 2 days, in the case of gross contamination, and 1-3 weeks under most circumstances. The results of virological tests on contaminated water are unlikely to be known before human illness occurs. Microbiological indicators may have a limited correlation with viral contamination, but they and chlorination afford more protection to public health than does any available alternative approach to routine monitoring of finished water.

Conclusions:

1. The presence of infective virus in drinking water is a potential hazard to the public health, and there is no valid basis on which a no-effect concentration of viral contamination in finished drinking water might be established.
2. Continued testing for viral contamination of potable water should be carried out with the facilities and skills of a wide variety of research establishments, both inside and outside the government, and methodology for virus testing should be improved.
3. The bacteriological monitoring methods currently prescribed or recommended in this report (coliform count and standard plate count) are the best indicators available today for routine use in evaluating the presence in water of intestinal pathogens including viruses.

Research Recommendations

1. Improved methods should be developed for recovery, isolation and enumeration of viruses from water supplies.
2. A laboratory method should be devised for detecting the virus of hepatitis A in potable water.
3. More should be learned about the etiology of viral gastroenteritis; special attention should be given to detection methods for gastroenteritis viruses that are transmissible through water.
4. The amount of virus that must be ingested in drinking water to produce infections and disease should be determined for several different enteric viruses.
5. Additional research should be conducted on the ability of various water-treatment methods to remove or inactivate viruses. Low-cost modifications to increase the reliability and effectiveness of existing methods should be sought.

Pathogenic protozoa and helminths

Man is the natural host of a number of parasitic animals. Among these, eggs and cysts of parasitic protozoa and helminths are deposited in the environment with excreta, and may enter water supplies. Water-purification processes have not been developed on the basis of considerations related to these organisms; but, fortuitously, the introduction of filtration, flocculation, and chlorination of water have been successful in diminishing the extent to which water serves as a source of parasitic infection. Perhaps more than water treatment, sanitary sewerage systems have diminished the spread of various intestinal helminths. Eggs of these helminths are not easily killed in sewage-treatment processes (Newton *et al.*, 1948; Greenberg and Dean, 1958; World Health Organization, 1964). Since there is a tendency for helmirth eggs to be concentrated in sewage sludges, there is some concern when sludges are used in agriculture (Greenberg and Dean, 1958; World Health Organization, 1964). There are specific drinking-water problems with protozoan parasites, such as *Entamoeba histolytica*, the cause of amebic dysentery and amebic hepatitis, and *Giardia lamblia*, a flagellate about whose pathogenicity there is no longer any serious doubt (Moore *et al.*, Wolfe, 1975; Wanner *et al.*, 1963; Zamcheck *et al.*, 1963; and, Brandborg *et al.*, 1967). In the United States in 1974, *Giardia lamblia* was the causal agent for the waterborne-disease outbreak which had the largest number of cases. (Center for Disease Control, 1976a).

Attention will be focused here on the protozoan organisms that represent a threat to human health. The intestinal helminths will be considered, especially in terms of the characteristics that make then susceptible to elimination from sewage effluents, and that also make them unlikely to be found in raw or finished water.

Protozoa

Parasitic protozoa replicate in the human host, and may be responsible for severe disease. *Entamoeba histolytica* (Craig, 1934; World Health Organization, 1969) has been found to be responsible for severe outbreaks of dysentery. It is also capable of setting up chronic infections in the human host, with the eventual development of abscesses of the liver and occasionally other organs. *Giardia lamblia* is not so severe a pathogen, but is responsible for gastrointestinal disturbances, flatulence, diarrhea, and discomfort. Both these organisms are able--on occasion--to penetrate our sanitary barriers.

Amebiasis.

During the period 1946-1970, there were five reported outbreaks of amebiasis due to *Entamoeba histolytica* transmitted by water. Four of these outbreaks were related to private distribution systems, and involved a total of 50 clinical cases. The well-studied South Bend outbreak in 1953, in which there were at least 750 infections and 30 clinical cases, with 4 fatalities, was due to sewage contamination of a private water supply to a factory (LeMaistre *et al.*, 1956). One outbreak involved a public system, and accounted for 25 cases (Craun and McCabe, 1973). Before 1946, there were two major amebiasis outbreaks due to contaminated water. The well-known Chicago epidemic of 1933 was traced to cross-connections between sewage and water lines in a hotel (Bundesen *et al.*, 1936). The Mantetsu-apartment-building outbreak in 1947 (Ritchie and Davis, 1948) in Tokyo was similarly traced to sewage contamination of water due to faulty distribution systems.

Facultatively Parasitic Amebae.

In recent years, a relatively large number of cases of meningoencephalitis have been reported as caused by free-living, facultatively-parasitic amebae of the genera *Naegleria*, *Hartmanella*, and *Acanthamoeba*. Most of these cases have been related to swimming in fresh-water ponds or swimming pools. However, more recently (Baylis *et al.*, 1936; Robert and Rorke, 1973) *Naegleria fowleri* and *Acanthamoeba sp.* have been isolated from tapwater in association with cases of primary amebic meningoencephalitis. It is possible that the occurrence of these fresh-water amebae will increase in surface water because of the leaching of fertilizers from agricultural lands, and the other factors that are contributing to accelerated eutrophication of ponds and lakes. Viable cysts, identified as *Naegleria* and *Hartmanella* species, have been found in eight of 15 finished-water supplies in a survey of large-city supplies across the United States (Robert and Rorke, 1973). The cyst densities were low, about 10-15/gal. Little is known about the characteristics of the cysts and trophozoites of these organisms. It seems likely that the cysts will be at least as resistant to chlorine as those of the parasitic amebae and flagellates. Therefore, considerable emphasis must be placed on flocculation and filtration processes, in order to to remove these organisms from the waters in which they may be proliferating.

Giardiasis.

Giardiasis is emerging as a major waterborne disease. The largest outbreak of giardiasis in the United States occurred in Rome, N.Y. (Center for Disease Control, 1975). There were 395 cases of symptomatic giardiasis identified by stool examinations in the 7-month period from November 1, 1974, to June 7, 1975. A random household survey, conducted during one week in early May, 1975, identified 150 stool-positive cases of giardiasis among 1,421 persons. On the basis of the survey data, the attack-rate was 10.6%, and more than 4,800 persons may have been ill; a larger number probably harbored asymptomatic infections. Ten samples of the raw water were collected with a large-volume water sampler and were fed to 10 pathogen-free beagle puppies, of which two, fed from different samples, became infected. The Center for Disease Control's diagnostic parasitology laboratory identified one *G. lamblia* cyst in one of the water samples. The Rome water-supply system did not maintain a free chlorine residual, but used only combined residual disinfection of surface water. Information on this and other *Giardia* outbreaks is reported in Foodborne and Waterborne Disease Outbreaks, Annual Summary 1974, Center for Disease Control (1976a).

Veazie (1969) noted the occurrence of some 50,000 cases of gastroenteritis in Portland, from October, 1954 to March, 1955. Some of these cases were attributed to infection with giardiae. A report of this outbreak appeared in the form of a letter to the editor in the *New England Journal of Medicine*, after publication of the study on the Aspen outbreak (Moore *et al.*, 1969). Waterborne outbreaks of giardiasis in the United States numbered 14 between 1969 and 1975 and involved at least 700 diagnosed cases. Of these, 12 outbreaks occurred during the period 1971-1974 (Craun 1975) and involved over 300 people. This apparent increase in incidence may be due to greater awareness on the part of physicians; much publicity was accorded to outbreaks of *Giardia* infections among tourists in Leningrad, starting in 1970. According to Schultz (1975), a questionnaire survey of 1,419 travelers to the U.S.S.R. showed that 324 (23%) had acquired giardiasis. The mean time between entry into the U.S.S.R. and the appearance of symptoms was 14 days, and the illness lasted 6 weeks. Leningrad was identified as the site of infection, and attack rates were highest among travelers who drank tapwater.

All the U.S. outbreaks involving municipal systems, except for a large outbreak at Aspen, Colorado, in 1965-1966, have been associated with surface-water sources where disinfection was the only treatment. At Aspen, it is apparent that wells which served as a source of water for part of the community were contaminated from old, broken sewer lines that passed close to them (Moore *et al.*, 1969).

Helminths

In the United States the most important intestinal worms that are transmitted in drinking water include *Ascaris lumbricoides*, the stomach worm; *Trichuris trichiura*, the whipworm; *Ancylostoma* *duodenale* and *Necator americanus*, the hookworms; and *Strongyloides stercoralis*, the threadworm. All of these are nematodes, or roundworms. The stomach worm and whipworm are transmitted directly from one host to another by their eggs, after the eggs have had an opportunity to develop outside the body;the development involves the formation of an infective larva from the embryo contained in the egg. The hookworms live in the soil, grow, and undergo two molts, until they reach a stage which is infectious to man. They usually gain entry into their new host by penetrating the skin, and then wandering through the body until they mature in the intestinal tract. The threadworm, like the hookworm, produces an infective larva that invades through the skin. It has the option of passing through one or several free-living generations before producing infective larvae; therefore, it can expand the number of infective larvae derived from each egg.

In addition to the nematodes described above, there is one cestode, or flatworm, that takes up residence in the human intestinal tract when it can. This worm is *Hymenolepis nana*, the dwarf tapeworm. It is the only one of the cestodes infecting humans that has a direct life cycle; i.e., the egg serves as the infecting agent by the oral route. The larva and adult develop from the egg. The adult produces a string of proglottides, up to 200; eggs are produced in each. The gravid proglottides often rupture in the intestinal tract of the host, setting the eggs free. The eggs are immediately infective when passed in the feces.

All other important helminthic parasites of man require intermediate hosts for the development of larval stages infective to man. Except for the guinea worm, *Dracunculus medinensis*, which develops as an infective larva in the water flea, *Cyclops*, the ingestion of water is a minor factor in the spread of these parasites from host to host. *D. medinensis* is not endemic in the United States.

The eggs of the stomach worm and the whipworm measure 60 x 45 µm and 52 x 23 µm, respectively. Hookworm and threadworm eggs are similar in size. The egg of the dwarf tapeworm is about 48 x 37 µm. All of these eggs are denser than water. They are also of such a size that they can be entrapped in sewage-treatment plants and in the sand filters of water-purification plants. However, motile larvae of the hookworms and of the threadworm are capable of moving through sand filters in water-purification plants, as has been shown to be the case with a number of free-living nematodes. Like the free-living nematodes, and possibly to a greater extent because infective larvae are resistant to the environment and do not ingest food, the larvae of these parasitic worms can be expected to survive usual chlorination procedures.

All the parasites considered above are soil-transmitted parasites. It is possible that surface runoff during heavy rains can bring eggs or larvae into raw-water sources. In treatment, however, their large size practically ensures that they will be entrapped in flocculation and filtration processes. Viable hookworm and threadworm larvae, because of their mobility, might be able to traverse sand filters. Because of an enormous dilution effect, and because even the larvae tend to settle out in water, one would be hard-put to attribute a human infection with one of these parasites to a water source, other than wells polluted from the surface. One other factor should be mentioned: the worm burden acquired by a host is directly related to the number of infective eggs or larvae to which the host is exposed; there is no replication of the worms in the definitive host. Therefore, the odds against the establishment of a serious worm infection in a human via a water-distribution system are extremely high. It is unlikely that the transmission of helminthic infections via water systems is significant.

Free-living nematodes that ordinarily are found in soil are occasionally washed into river water by heavy rains, and can pass rapid sand-filtration barriers and survive chlorination. These nematodes belong to the genera *Cheilobus*, *Diplogaster*, *Trilobus*, *Aphelenchus*, *Rhabditis*, and others. They are not parasitic and pose no direct threat to man. However, they are reported to produce a gummy substance, small quantities of which confer an unpleasant taste to finished water. Besides having this characteristic, the nematodes may pose a problem in that they are resistant to chlorine; free residual chlorine at 2.5-3.0 ppm failed to immobilize them in 120 minutes (Chang *et al.*, 1961). Because these nematodes ingest bacteria, it is conceivable that they could serve to protect pathogenic organisms from chlorine and therefore take them to the consumer. However, the chances of this appear small, because the nematodes pass the bacteria when they are active, and shed bacteria if they molt to a sheathed (resistant) stage. As examples of actively motile nematode larvae, such as infective hookworm and *Strongyloides* larvae, these nematodes indicate the ease with which such organisms can penetrate sand filters. Statistically, however, the chances are small that water-distribution systems contribute significantly to the spread of parasitic nematode infections, or that viable free-living nematodes will carry any significant numbers of bacterial pathogens past the disinfection systems.

Water-Treatment Practices And Parasite Removal

Cysts of *Entamoeba histolytica* are resistant to high concentrations of chlorine (Brady *et al.*, 1943; Stringer *et al.*, 1975). Concentrations of chlorine required to kill cysts in raw water in 20-30 minutes can exceed 9 ppm, depending on pH and other conditions; certainly, residual-chlorine concentrations in water-distribution systems are not adequate. *Giardia* cysts are also not reportedly destroyed by chlorination at conventional doses and contact times. It has been stated that they will survive 0.5 ppm for 30 minutes. Ozone, applied at 0.5 ppm, can kill 97% to more than 99% of cysts of *E. histolytica* suspended in tapwater (Newton and Jones, 1949). Bromine is a more effective cysticide than chlorine or iodine. At a pH of 6, a bromine residue at 1.5 ppm was needed to produce 99.9% cyst mortality in 10 minutes. The same degree of cyst-destruction was achieved by iodine residue at 5 ppm and chlorine residue at 2 ppm, respectively. High pH's decrease the cysticide activity of all three halogens (Stringer *et al.*, 1975); however, the cysts of both these protozoans are of such size that flocculation and filtration can be expected to remove them from finished water (Anderson *et al.*, 1973). The cyst of *E. histolytica* measures 10-15 µm in diameter, and that of *G. lamblia* measures 9-12 x 6-9 µm. When outbreaks of *G. lamblia* have been traced to municipal water, the system has relied on disinfection, rather than including flocculation and filtration. This suggests that mechanical means of clarifying water are important as antiparasitic measures. Because the protozoan cysts are resistant to usual residual-chlorine concentrations maintained in the distribution system, the need for filtration is emphasized. It is also important to emphasize the danger of faulty distribution systems, even when relatively high residual-chlorine concentrations are maintained; cross-connection control is essential.

Conclusions

The principal pathogenic parasites that may escape our sanitary barriers in public water supplies are the protozoa *Entamoeba histolytica* and *Giardia lamblia*. The cysts of these organisms are not completely destroyed by the usual chlorination. Most cysts, however, will be removed by sedimentation and filtration through sand. Filters should be of adequate depth, and the rates of filtration and back-flushing should be adjusted, to ensure entrapment of cysts and to prevent turnover of the filter or channeling. Proper maintenance is essential. The greatest risks will occur in water systems that use only disinfection, and in contamination of water in the distribution system by sewage from broken lines or from cross-connections. Normal residual chlorine in such circumstances cannot kill cysts.

More study is needed to define the conditions required for destruction of *Giardia lamblia* cysts; little is known of their survival.

Occasionally, facultatively-parasitic amebas can pass through the treatment processes and appear in finished water. Although these amebas can produce fatal encephalitides in man, the usual route of entry is the nasal passages, while a person swims in a pool or a pond of untreated or inadequately-treated fresh water. The numbers of free-living ameba cysts found in finished water are small.

Entrapment of pathogenic bacteria by parasites and other metazoa, such as free-living nematodes, may lead to protection of the bacteria from disinfection. However, it appears unlikely--on the basis of the statistics of the occurrence of such organisms in finished water, the condition of the organisms seen, and the fact that viable nematodes are continually passing bacteria through their gut--that a significant number of pathogenic organisms would survive in finished water with an adequate residual disinfectant.

MICROBIOLOGY OF DRINKING WATER

SUMMARY

The incidence of enteric disease in the United States has been reduced by effective water treatment systems, but in 1974 the Center for Disease Control reported 28 waterborne disease outbreaks and 8,413 cases. In 1975 there were 24 outbreaks and 10,879 cases.

Very few of these outbreaks were caused by chemical poisoning. Most cases (6,832 or 81%) in 1974 were caused by microorganisms. The category designated "acute gastrointestinal illness" accounted for the largest number of cases (9,760) in 1975. These cases were characterized by symptoms for which no etiologic agent was identified. They account for approximately 90% of the total cases reported by the Center for Disease Control in 1975. Microorganisms account for most of the remaining cases.

Improved detection and reporting systems are needed to determine more accurately the incidence of waterborne diseases nationwide. Outbreaks occur that are not reported. The etiologic agent was not identified in 15% of the cases reported in 1974, and 90% of those reported in 1975. Improved alarm systems are needed.

In 1971-1974, deficiencies in treatment, such as inadequate or interrupted chlorination and contamination of ground water, were responsible for a majority (65%) of the waterborne-disease outbreaks. In 1975, treatment deficiencies were responsible for most outbreaks; however, deficiencies in the distribution systems were responsible for the highest number of cases.

BACTERIA

The control of waterborne epidemics still depends largely upon the control of infectious enteric diseases. Much of the success in this regard can be attributed directly to the use of chlorine as a disinfectant. Although the excessive use of chlorine in water treatment may result in the formation of several compounds that are known carcinogens for animals, and suspected carcinogens for humans, the advantages far outweigh this limitation.

Several substitutes for chlorine (e.g. ozone, chlorine dioxide, bromine and iodine) that are also powerful oxidants and disinfectants have been suggested, but much more research is required before any of them can be recommended as a sole substitute for chlorine in water treatment. Questions concerning disinfection effectiveness, toxicity of by-products, and residual in the distribution system, must be answered for proposed substitutes as well as for chlorine. It may be possible to reduce the concentrations of undesirable organic by-products of chlornation--without compromising disinfection--by changing the sequence or rate of chlorine addition in relation to other steps in water treatment. Partial use of other oxidizing agents, before chlorination, may also help to modify organic matter before significant amounts of chlorinated derivatives can be formed.

Good engineering and public health practices emphasize the need for using raw water of the highest possible quality. Bacteriological testing, or the imposition of bacteriological standards, are adjuncts to, not substitutes for, good quality raw water, proper water treatment, and integrity of the distribution system. Application of the present coliform standards appears adequate to protect public health when raw water is obtained from a protected source, is appropriately treated, and is distributed in a contamination-free system.

Current coliform standards are not satisfactory for water reclaimed directly from wastewater. The meeting of current coliform standards is insufficient to protect public health for water reclaimed directly from waste water, or for water containing several percent of fresh sewage effluent. Additional microbiological standards for such situations, possibly based on the content of viruses, Clostridia, or total bacteria in the finished water, should be developed and applied as a supplement to coliform standards.

The standard plate count is not a substitute for total coliform measurements of the sanitary quality of potable water; it is, however, a valuable procedure for assessing the bacterial quality of drinking water. Ideally, standard plate counts (SPC) should be performed on samples taken throughout the systems. The SPC has major health significance for surface-water systems that do not use flocculation, sedimentation, filtration, and chlorination, and for those ground-water systems that do not include chlorination.

VIRUSES

The bacteriological monitoring methods currently prescribed (coliform count, standard plate count) are the best indicators available today for routine use in determining the probable levels of viruses in drinking water. The best available water treatment technology, if diligently applied, should provide a high degree of assurance that viruses injurious to human health are absent from finished drinking water. However, because knowledge of the scale of potential viral contamination is scanty, and because there is no rigorous basis for establishing a harmless level of viral concentration in water, research on the problems of viral contamination should be strongly supported. In particular, the following subjects deserve special attention:

1. methods for testing of potable water for viral contamination
2. methods for recovery, isolation, and evaluation of viruses (especially hepatitis A)
3. specific etiology of viral gastroenteritis
4. the amount of virus that must be ingested to produce infections and disease should be determined for several different enteric viruses
5. methods for evaluating and improving effectiveness of present water treatments to remove or inactivate viruses.

PARASITES

The most important waterborne parasitic diseases in the United States are amoebiasis and giardiasis. Outbreaks of amoebiasis appear to have resulted from sewage contamination in the distribution systems. Giardiasis, which in recent years has become a major problem in some areas, appears to be associated with inadequately-treated surface waters.

The cysts of both of these parasites are more resistant to chlorine than are bacteria, but flocculation and filtration can remove them. Nevertheless, knowledge of the vulnerability of all of these organisms to disinfection is incomplete, and in particular, the conditions necessary for destruction of Giardia cysts require further study. The same considerations apply to a few other parasitic protozoa that, although rare, have been identified in public water systems.

Metazoan parasites (helminths, nematodes) that can be present in raw water will be controlled in public water supplies by well-regulated flocculation, filtration, and disinfection.

TESTING

One of the greatest deficiencies of customary methods for evaluating the bacteriological quality of water is that results from tests are unknown until after the sampled water has already entered the distribution system and been used. Successful regulation of the microbiological quality of drinking water therefore depends on the use of raw water supplies of relatively invariant high quality. Sudden invasions of contamination are unlikely to be detected promptly enough to prevent exposure, and in addition, may overwhelm the corrective treatments.

Nevertheless, it is essential that present methods of microbiological testing be continued, in order to validate the effectiveness of disinfection, and for detecting defects within the system.

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CHAPTER IV

SOLID PARTICLES IN SUSPENSION

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Chapter IV

SOLID PARTICLES IN SUSPENSION

Introduction

In addition to dissolved substances, drinking water typically contains small amounts of very finely divided solid particles of several kinds. These particles, ranging in size from colloidal dimensions to about 100 micrometers, are composed of inorganic and organic materials that are derived from soils and rocks, and from the debris of human activity with which the raw water has come in contact. They include clays, acicular of fibrous particles of asbestos minerals, and organic particles resulting from the decomposition of plant and animal debris in the soil.

Little is known about the effects that these suspended solids may have on the health of those who drink water that contains them. However, there is widespread concern over the biological effects of the asbestos mineral fibers that occur in water, since similar fibers are known to be carcinogenic when air heavily laden with them is inhaled for many years. In view of this concern that such fibers as occur in water may be injurious to health, their occurrence, characterization, analysis and biological effects are reviewed in some detail.

No evidence has yet been discovered that either of the other classes of common particulate contaminants of drinking water - clays and organic colloids - has any direct effect on health. Nevertheless, it is possible that both may indirectly affect the quality of drinking water because they can adsorb a variety of toxic substances, bacteria and viruses from solution or suspension and bind them more or less strongly. By such means these materials may serve to concentrate and transport some water pollutants and protect them from removal by water treatment.

For this reason, the properties of clays and organic particulates are also discussed, together with the tendency of chemicals, bacteria, and viruses to become concentrated at the surfaces of such particles.

Removal of suspended particles from water is briefly reviewed, together with the significance of measurements of turbidity as an index of water quality.

CLAY PARTICLES AND THEIR INTERACTIONS

Clay is usually defined on a particle-size basis, the upper limit being 2 micrometers diameter. Soils and sediments in nature will therefore have varying proportions of clay material containing clay-mineral components (usually the phyllosilicates), as well as nonclay-mineral material, that can be a variety of substances such as iron and aluminum oxides and hydroxides, quartz, amorphous silica, carbonates, feldspar. The clay minerals themselves are classified in Table IV-1 (Grim, 1968).

Clays are ubiquitous in soils and sediments derived from soils. They may be formed in soils during soil development through the weathering of various minerals, or they can be inherited essentially without change from the parent material upon which the soils are formed. Parent material, climate, topography, and vegetation determine the kinds of clays that are found. Other environments leading to clay formation are hydrothermal types of activity. As erosion acts on the landscape, clays may be suspended in water and carried until they are deposited by sedimentation. Most sedimentary rocks contain more or less clay as, for example, shales (almost exclusively clay), limestones, and sandstones.

A number of scientific techniques are useful for studying clays, however the most useful for identification and indication of relative abundance is x ray diffraction. The diffraction properties of the various clay minerals as well as the methods of treatment and sample preparation can be found in publications of Grim, 1968; Brown, 1961; and Whittig, 1965. Infrared spectroscopy is a valuable adjunct to X-ray diffraction in characterizing clays, and this subject has recently been reviewed by Farmer, 1975. Infrared spectroscopy is the most powerful method for study of organic-clay interactions (Mortland, 1970; Theng, 1975). Other techniques useful in characterizing clays are electron microscopy (Gard, 1971), thermal methods (Mackenzie, 1957) and chemical analysis (Weaver and Pollard, 1973).

Table IV-1

Classification of the Clay Minerals (Grim, 1968)

I. Amorphous

Allophane group

II. Crystalline

A. Two-layer type (sheet structures composed of units of one layer of silica tetrahedrons and one layer of alumina octahedrons)
1. Equidimensional
Kaolinite group
Kaolinite, nacrite
2. Elongate
Halloysite group

B. Three-layer types (sheet structures composed of two layers of silica tetrahedrons and one central dioctahedral or trioctahedral layer)
1. Expanding lattice
a. Equidimensional
Montmorillonite group (smectite)
Montmorillonite, sauconite
Vermiculite
b. Elongate
Montmorillonite group
Nontronite, saponite, hectorite
2. Nonexpanding lattice
Illite group

C. Regular mixed-layer types (ordered stacking of alternate layers of different types)
Chlorite group

D. Chain-structure types (hornblende-like chains of silica tetrahedrons linked together by octahedral groups of oxygens and hydroxyls containing Al and Mg atoms)
Attapulgite
Sepiolite
Palygorskite

The layer-lattice clay minerals, in themselves, do not appear to have deleterious effects when ingested by humans. Some of them are, in fact, constituents of pharmaceuticals such as kaopectate (kaolinite). Other indications (some from folklore) suggest beneficial results from ingestion of clays. The effect of ingeston of fibrous clay minerals of the chain-structure types (e.g., attapulgite, palygorskite, sepiolite, sometimes called "asbestos"), is still open to question and is the subject of extensive study at the present time. If layer-lattice clay minerals have deleterious effects on human health, they are probably indirect, through adsorption, transport, and release of inorganic and organic toxicants, bacteria, and viruses.

Several reports have shown that concentrations of many pollutants are much higher in sediments of streams and lakes than in the waters with which they are associated. Clays and organic particulates are the materials chiefly responsible for such concentrations. Since clays are ubiquitous in many waters used as sources for human consumption, it is to be expected they will appear as particulate matter in some drinking waters and thus it is of interest to consider the kinds of interactions they have with dissolved materials. Considerable knowledge exists regarding the surface chemistry and adsorptive properties of clays, and thus, with information on the nature of a solute, some idea of their interaction is possible. Clays are very adsorptive substances. The possibility exists that clays could act as vehicles for transport of toxic compounds through adsorption in one environment followed by release of the toxic material when the clay entered a different environment.

It has been well established that some pesticides applied to watersheds can be adsorbed by soil components and subsequently removed into water by erosional processes (Bailey et al., 1974; Nicholson, 1969; Nicholson and Hill, 1970).

Inorganic Pollutants

This classification of pollutants would include metal cations and some anions. Among the metal cations that have been found to be polluting some water and soils are Pb, Cr, Cu, Zn, Co, Mn, Ni, Hg, Cd, while radioactive isotopes of Pu, Cs, Sr, among others offer potential threats as pollutants. On the other hand, anionic species such as phosphates, arsenate, borate and nitrates are considered pollutants in some situations.

The interactions of metal cations with clays include adsorption by ion exchange, precipitation as hydroxides or hydrous oxides on clay surfaces, and adsorption as complex species. Obviously, pH and Eh are critical factors in determining the nature of the interactions between clays and some transition and heavy metal ions. Hodgson's review (1963) includes some reference to earlier work on clay interactions with some of the transition ions and heavy metals. Jenne (1968) has effectively described the various factors controlling the concentrations of transition cations in waters and soils, while Jenne and Wahlberg (1968) and Tamura (1962) among others have considered the interaction of radionuclides with clays. Holdridge (1966) has reported adsorption studies of heavy metal cations on ball clay. With regard to phosphate, it is likely its interactions with calcium ion and amorphous hydroxides of Fe^{3+} and Al^{3+} and with allophane are more important than its adsorption by clay minerals in affecting its concentration in natural waters.

In addition to adsorption by simple ion exchange, much work indicates the retention of transition and heavy metals at clay mineral surfaces via precipitation of insoluble compounds, notably hydroxy and oxidehydroxy polymers. The incorporation of Al^{3+} Mg^{2+} and Fe^{3+} hydroxy polymers within the interlamellar space of swelling clays to form chlorite-like species is a well known pedogenic process. It has also been shown that these brucite-gibbsite like materials may often be withdrawn if the mineral is subjected to a different environment, usually one involving a change in pH. Gupta and Malik (1969) have reported the incorporation of Ni^{2+} in smectite to form a Nickel-chlorite, while Blatter (1973) found similar reactions of smectite with Hg^{2+}. Thus it seems that many of these kinds of metal cations have the ability to form interlayer complexes in swelling clays. It would seem likely that in natural systems where the polluting species might be present in very low concentrations compared with other interlayer-forming species such as Al^{3+}, that might be incorporated within the gibbsite-like layer as it forms, in essentially isomorphous substitution for Al^{3+}, although reports of this phenomenon were not found. It would also appear that incorporation of a polluting metal cation within the intergrade clay is no guarantee that it might not again be released to the natural system when the clay, through erosion and deposition, is placed in a different environment where the interlayer material may be removed. This phenomenon has been shown for vermiculite-chlorite intergrades which, upon erosion from an acidic soil, are deposited in a calcareous fresh water lake or flood plain to form discrete vermiculite, within relatively short periods of time (Frink, 1969 and Lietzke and Mortland, 1973).

The clay mineral vermiculite has a special affinity for K^{+} ion in which the ion is initially adsorbed in the interlamellar regions of the mineral and then trapped by collapse of the layer structure. The ion is thus removed from direct interaction with the surrounding solution. This process is called potassium fixation, but will occur with other ions of similar diameter to more or less extent. Cations that might be considered pollutants that undergo this reaction with vermiculite are Ba^{2+} and radioactive Cs^+.

The hydroxides and hydrous oxides of iron, manganese, and aluminum are often components of the clay fraction of sediments and have important effects on pollutant concentrations in natural waters. They often exist as coatings on the surfaces of other minerals and thus may exert chemical activity far out of proportion to their total concentrations. Jenne (1968) suggests that they furnish the principal control on the concentrations of heavy metals such as Co, Ni, Cu, and Zn through adsorption processes. The principal factors affecting adsorption and desorption of heavy metals from these kinds of particulates are pH, Eh, concentration of the metal in question, concentration of competing metals and the effects of other adsorbents such as organic matter and clay minerals.

Organic Pollutants

These materials encompass a wide range of compounds including pesticides, polychlorinated biphenyls, aromatic species of various kinds arising from industrial activity, fluorine compounds in aerosols, etc. Whether or not organic species adsorb or interact with clays depends upon the structure and properties of the compound and the nature of the clay and its exchangeable cations. Several mechanisms of interaction are possible, and have been described in a number of recent reviews (Mortland, 1970; Bailey and White, 1970; Theng, 1974; and Rausell-Colom and Serratosa, 1975). Organic cations adsorb on clays by ordinary ion exchange and are usually preferred over the inorganic ions by the exchange complex because of their large size and high molecular weights. Examples of organic compounds that are cationic, and could be considered pollutants if transported outside their areas of application, are the herbicides Paraquat and Diquat. These compounds are strong bases and are completely ionized in water. Other organic compounds, while neutral at the ambient pH of the solution phase, may become protonated after adsorption at the clay surface. The surface acidity of clays has been shown to be a considerably stronger proton donor system than pH measurements of the water-clay system would indicate. Thus, organic compounds containing basic nitrogen or carbonyl groups may become protonated, and therefore cationic, after adsorption at clay surfaces. Another kind of organic-clay interaction is the coordination or ion-dipole type. Compounds with nitrogen, oxygen, sulfur, or olefinic groups have electron pairs that may be donated to electrophilic exchange cations to form complexes on the clay surface. In natural systems, an important consideration is the competitive effect of water for these adsorption sites. That is, the energy of ligand formation of an organic molecule with an exchange cation must be greater than the solvation energy of the cation in order to displace water molecules and obtain direct organic-cation coordination. In the laboratory these interactions are easily obtained by dehydration; however, in natural systems the competition of water is a major factor in determing whether or not these complexations occur. Nevertheless, it is likely that this kind of interaction does occur for some highly polar, electron donating organic compounds. Another important factor is the nature of the exchange cation. Thus, for example, transition metal cations on the exchange complex, that have unfilled d orbitals, will interact strongly with electron-supplying groups of organic molecules. Still another kind of organic-clay interaction is hydrogen bonding. These can be classified into three types: 1) hydrogen bonding between water molecules directly solvating exchangeable cations and polar functional groups, such as carbonyl, on organic molecules. The water molecules thus act as a "bridge" between the cation and organic species. 2) Hydrogen bonding between functional groups such as alcoholic and amino groups and oxygens of the silicate surfaces. Infrared spectroscopy has indicated that those are relatively weak bonds, being within the lower range of energies where hydrogen bonding is found. 3) Intermolecular hydrogen bonding between two organic species on the clay surface. Other factors involved in clay-organic interactions include physical forces and entropy effects.

With this general description of the interactions of organic species with clays, it is now appropriate to mention some special clay-organic properties that have relevance to organic pollutants. Many organic compounds, including aromatics and particularly the halogenated types such as DDT, chlorinated and brominated phenyls and biphenyls, etc., are adsorbed to little if any extent on clay surfaces from aqueous solution. In the natural environment they are more likely to be adsorbed in organic components of soils and sediments. These materials usually have limited solubility in water, since they are hydrophobic. It is thus not surprising that they are not attracted to the hydrophilic surfaces of clays. The above discussion, however, suggests that, in natural systems, clay-organic complexes may act as adsorbing media for some organic pollutants that are not adsorbed at all by pure inorganic clays.

Another phenomenon that may take place when organic species are adsorbed at clay surfaces is that of catalytic alteration. This has particular relevance for organic pollutants since there is much interest in their fate in the environment. Much work has been reported on catalytic reactions on clays at high temperatures, but it is only recently that much attention has been paid to catalysis by clays in conditions resembling the natural environment. One mechanism by which clays can act as catalysts is via their Bronsted acidity. Examples of this are the hydrolysis of esters demonstrated by McAuliffe and Coleman

(1955), the conversion of atrazine to hydroxyatrazine by Russell et al. (1968), the decomposition of alkylammonium ions by Chaussidon and Calvet (1965), and the hydrolysis of nitriles to amides by Sanchez et al. (1972). In many decomposition reactions involving Bronsted acidity, carbonium ion formation is undoubtedly involved. On the other hand, Lewis acid sites may exist in clays that also will catalyse many organic reactions. These sites (electron acceptors) may be part of the basic structure of the mineral itself as, for example, ferric iron within the octahedral layer or exposed aluminum on the edges of the minerals. In addition, some cations on exchange sites function in this capacity, particularly those of the transition metal ion group. Solomon et al. (1968) have demonstrated catalytic properties of Lewis sites located on edges of clay minerals. The activity of some transition metal cations on exchange sites has also been amply demonstrated as, for example, the decomposition of urea when complexed with Cu^{2+}, Mn^{2+}, or Ni^{2+} smectite, to ammonium ion. No such reaction was noted for urea complexed with alkali metal or alkaline earth saturated clay (Mortland, 1966). Aromatic molecules such as benzene will complex via pi electrons with clay minerals saturated with Cu^{2+}, under mild desiccating conditions. Under more vigorous dehydrating conditions, a radical cation of benzene is formed that will react with molecular benzene to give polymers containing phenyl groups as well as fragmented benzene rings (Doner and Mortland, 1969). Anisole (methoxybenzene) will also form radical cations that react with molecular anisole to give 4,4'-dimethoxybiphenyl (Fenn et al., 1973). Other cationic species on clays with oxidizing abilities as great as Cu^{2+} such as VO^{2+} and Fe^{3+} were also found to produce radical cations from some aromatic species with subsequent polymer formation (Pinnavaia et al., 1974). These reactions suggest the possibility that some pollutant species adsorbed on clay surfaces may undergo similar reactions to form radical cations and subsequently interact with themselves or other organic compounds present with formation of different chemical derivatives. Thus, pollutant degradation or alteration on clays by oxidation-reduction reactions involving exchangeable transition-metal cations may be a real possibility in nature.

In addition to the degradation of atrazine to hydroxyatrazine, mentioned above, a number of other clay-catalysed pesticide reactions have been reported. For example, Fleck and Haller (1945) report the conversion of DDT to DDE by kaolinite and smectite samples, preheated to 400°K. Also, degradation of heptachlor by palygorskite has been suggested by Malina *et al.* (1956). The degradation of the organic phosphate insecticide, ronnel, by clays heated to various temperatures has been reported by Rosenfield and van Valkenburg (1965). Organic phosphate pesticides have been observed by Mortland and Raman (1967) to be hydrolysed in the presence of Cu^{2+}-montmorillonite by a coordination mechanism. The much weaker catalytic effects of Cu^{++}-vermiculite, beidellite, and nontronite were attributed to reduced activity of the copper on these minerals as compared with montmorillonite, due to charge location. While most of the degradation of pesticides in nature has been attributed to biological agencies, the above discussion would suggest that catalysis at mineral surfaces may also play a role.

Natural organic material in soils forms complexes with clays that exert important influences on the physical, chemical, and biological properties of the soil (Greenland, 1965, 1971). Since the exact chemical and physical nature of these organic materials is not known, the kinds of interaction they have with clays are less well known than that of well defined organic compounds. However, some of the kinds of reactions described above are probably involved. It is obvious that clays eroded from soil surfaces into streams and lakes will probably be, to some degree, complexed with organic matter.

Humic acids, a constituent of soil organic matter, may be strongly adsorbed by clays, presumably by interaction with positive sites on the edges of clay particles, or with polyvalent cations on the cation exchange complex acting as "bridges". Schnitzer and Kodama (1967) have shown that fulvic acid (another constituent of soil organic matter) adsorption depends on pH, and is greater under acid than alkaline conditions. This is to be expected since the fulvic acid would be relatively undissociated at low pH but considerably more anionic at alkaline pH. Schnitzer and Kodama (1972) showed that fulvic acid is very strongly bound to Cu^{++} on the exchange sites of montmorillonite through a coordination type of reaction. In addition they have shown such adsorption is typical for any electrophilic cation on the exchange complex, particularly for ions of the transition metal group.

Summary

Pollutant concentrations are higher in sediments than in the waters with which they are associated. It should be recognized that the consequences of pollutant adsorption by clays may be very important in natural systems and may affect drinking water quality. Clay-pollutant complexes may be mobilized by erosion from the landscape, or form when eroded clay enters a stream containing a polluting species. If the complex survives water treatment and enters the drinking water system, it would then be available for ingestion by humans. In the adsorbed state on the clay surface the pollutant is probably not toxic, but the possibility exists that the pollutant might be released from the clay in the environment of the alimentary tract and thus exert toxic effects. Whether or not such a process might take place would depend on the complex in question so that no generalities are possible. Information is completely lacking in this area, and thus research should be encouraged and supported.

ASBESTOS: NOMENCLATURE, OCCURRENCE AND REDISTRIBUTION IN WATER

Structure and Nomenclature

Asbestos is the name for a group of naturally occurring hydrated silicate minerals possessing a fibrous morphology, and commercial utility. This definition generally limits application of the term to the minerals chrysotile, some members of the cummingtonite-grunerite series, crocidolite, anthophyllite, and some members of the tremolite-actinolite series. "Amosite" is commonly used in reference to a cummingtonite-grunerite asbestos mineral but is a discredited mineral name (Rabbit 1948, and Committee on Mineral Names, 1949).

Mode of occurrence and fibre length are important determinants of commercial value. Of the commercially mined and processed asbestos minerals chrysotile accounts for about 95%, the remainder being "amosite" and crocidolite (May and Lewis 1970). Crocidolite is the fibrous equivalent of riebeckite, and chrysotile belongs to the serpentine group of minerals, which contains other non-fibrous members. (Deer *et al.*, 1970). Non-commercial deposits of asbestos minerals are also relatively common.

The standard definitions of the Glossary of Geology (American Geological Institute, 1972, Second Printing 1974) are given below.

"ASBESTOS: (a) a commercial term applied to a group of highly fibrous silicate minerals that readily separate into long, thin, strong fibers of sufficient flexibility to be woven, are heat resistant and chemically inert, and possess a high electric insulation, and therefore are suitable for uses (as in yarn, cloth, paper, paint, brake linings, tiles, insulation cement, fillers, and filters) where incombustible, nonconducting, or chemically resistant material is required. (b) a mineral of the asbestos group, principally chrysotile (best adapted for spinning) and certain fibrous varieties of amphibole (ex. tremolite, actinolite, and crocidolite). (c) a term strictly applied to the fibrous variety of actinolite. Syn: asbestus; amianthus; earth flax; mountain leather.

ASBESTIFORM: Said of a mineral that is fibrous, i.e. that is like asbestos.

ACICULAR (Cryst): Said of a crystal that is needlelike in form. Cf: fascicular, sagenitic

FIBROUS: Said of the habit of a mineral, and of the mineral itself (e.g. asbestos), that crystallizes in elongated thin, needle-like grains, or fibers."

The nomenclature used in this report conforms generally to these definitions, subject only to the further qualifications that the term asbestos will not be used in its most restrictive sense (c, above); asbestiform will not be used; and the terms acicular and fibrous are to be understood as discussed below.

The term asbestos has often been used in recent scientific literature to describe individual fibrous or acicular particles of microscopic and submicroscopic size. However, mineralogists and geologists have hastened to point out that the term should be used only as defined above, in reference to the minerals in bulk. Ampian (1976) considers the terms asbestos and asbestiform to be synonymous and that they then may only be used to apply to the bulk fibrous forms occurring in nature.

Asbestiform is often used to define the morphology of a mineral that is similar to asbestos, but does not necessarily occur in nature in a commercial deposit; to avoid ambiguity, the term will not be used here.

The terms acicular and fibrous are used here to characterize any mineral particle that has apparent crystal continuity, a length-to-width aspect ratio of 3 or more and widths in the micrometer or sub-micrometer range. Although the two terms are not strictly synonymous, the use here of either one to describe a mineral particle should be taken to imply the other, unless otherwise qualified.

Table IV-2 lists some of the naturally occurring minerals that can have, but do not always have, an acicular morphology. To this list could be added a number of synthetic fibers, although they are not naturally occurring minerals. Many of the minerals in Table IV-2 are common rock-forming minerals.

Properties of asbestos minerals

Mineralogy: The asbestos minerals belong in the serpentine and amphibole groups, and the amphiboles are further divided into those of the orthorhombic crystal system (orthoamphiboles) and amphiboles of the monoclinic crystal system (clinoamphiboles). Table IV-3 summarizes the basic properties of the asbestos minerals.

Chrysotile is the asbestos mineral in the serpentine group. Its crystal structure is a double sheet, comprising a layer of silica tetrahedra and a layer of magnesia octahedra, arranged in a manner that is somewhat analogous to the alumina octahedra - silica tetrahedra layering of kaolinite. The way in which the sheet structure is modified to develop a fibrous morphology in,

TABLE IV-2. List of some minerals that may occur in acicular-fibrous form. Major element stoichiometry has been simplified in many cases.

Silicates	
Epidote	$Ca_2FeAl_2O \cdot OH(Si_2O_7)(SiO_4)$
Lawsonite	$CaAl_2(OH)_2Si_2O_7 \cdot H_2O$
Sillimanite (fibrolite)	Al_2SiO_5
Pectolite	$Ca_2NaH(SiO_3)_3$
Kyanite	Al_2SiO_5
Mullite	$3Al_2O_3 \cdot 2SiO_2$
Diopside-hedenbergite	$Ca(Mg,Fe)(Si_2O_6)$
Enstatite	$MgSiO_3$
Wollastonite	$Ca(SiO_3)$
Jadeite (nephrite)	$NaAl(Si_2O_6)$
Anthophyllite	$(Mg,Fe)_7(Si_8O_{22})(OH,F)_2$
Tremolite-actinolite	$Ca_2(Mg,Fe)_5(Si_8O_{22})(OH,F)_2$
Hornblende	$(Ca,Na,K)_{2-3}(Mg,Fe^{2+},Fe^{3+},Al)_5(Si_6(Si,Al)_2O_{22})(OH,F)_2$
Crocidolite (glaucophane-riebeckite)	$Na_2Fe_3Fe_2(Si_8O_{22})(OH,F)_2$
Cummingtonite-grunerite	$(Fe,Mg)_7(Si_8O_{22})(OH)_2$
Eckermannite-arfvedsonite	$Na_3(Mg,Fe)_4Al(Si_8O_{22})(OH,F)_2$
Halloysite	$Al_2Si_2O_5(OH)_4$
Nontronite	
Palygorskite-attapulgite-sepiolite	$(Mg,Al)_5Si_8O_{22}(OH)_4 \cdot 4H_2O$
Chrysotile (serpentine)	$Mg_3Si_2O_5(OH)_4$
Talc	$Mg_3Si_4O_{10}(OH)_2$
Prehnite	$Ca_2Al(AlSi_3O_{10})(OH)_2$
Natrolite	$Na_2(Al_2Si_3O_{10}) \cdot 2H_2O$
Scolecite	$Ca(Al_2Si_3O_{10}) \cdot 3H_2O$
Thomsonite	$NaCa_2(Al_5Si_5O_{20}) \cdot 6H_2O$
Stilbite	$(Ca,Na_2,K_2)(Al_2Si_7O_{18}) \cdot 7H_2O$
Mordenite	$(Na_2,K_2,Ca)(Al_2Si_{10}O_{24}) \cdot 7H_2O$
Ferrierite	$(Na,K)_2Mg_2(Al_6Si_{30}O_{72})(OH)_2 \cdot 18H_2O$
Non-silicates	
Cassiterite	SnO_2
Goethite-limonite	$FeO(OH) \cdot nH_2O$
Rutile	TiO_2
Nemalite (brucite)	$Mg(OH)_2$
Stibnite	Sb_2S_3
Bismuthinite	Bi_2S_3
Marcasite	FeS_2
Jamesonite	$2PbS \cdot Sb_2S_3$
Gypsum	$CaSO_4 \cdot 2H_2O$
Celestite	$SrSO_4$
Alum	$KAl(SO_4)_2 \cdot 12H_2O$
Aragonite	$CaCO_3$
(Calcite)	$CaCO_3$
(Siderite)	$FeCO_3$
Apatite	$Ca_5(PO_4)_3(OH,F)$
Vivianite	$Fe_3(PO_4)_2 \cdot 8H_2O$

TABLE IV-1

MINERALOGY OF COMMON ASBESTOS MINERALS

Name	Chrysotile	Anthophyllite	Cummingtonite-Grunerite)	Crocidolite (Glaucophane-Riebeckite)	Actinolite Tremolite
Group	Serpentine	Amphibole	Amphibole	Amphibole	Amphibole
Structure	Sheet silicate	Double chain	Double chain	Double chain	Double chain
Composition	$Mg_3(Si_2O_5)(OH)_4$ constant (trace Ni, Cr, Al, Fe^{+2}, Fe^{+3})	$(Mg,Fe)_{5-6}Al_{1-2}(Si,Al)_8O_{22}(OH,F)_2$	$(Mg,Fe)_7(Si_8O_{22})(OH)_2$ to $Fe_7(Si_8O_{22})(OH)_2$ $(Mg,Fe)_7(Si_8O_{22})(OH)_2$ Mn common substitute	$Na_2Fe_3^{+2}Fe_2^{+3}(Si_8O_{22})(OH,F)_2$ Mg substitutes for Fe^{+2} Ca for Na	$Ca_2(Mg,Fe^{+2})_5(Si_8O_{22})(OH,F)_2$ $Ca_2Mg_5(Si_8O_{22})(OH,F)_2$ Al substitutes often; see hornblende
Cleavage	Perfect basal cleavage	(210) perfect	(110) good $(110) \wedge (1\bar{1}0) = 55°$	(110) good $(110) \wedge (1\bar{1}0) = 56°$	(110) good $(110) \wedge (1\bar{1}0) = 56°$
Crystal System	Monoclinic Cm	Orthorhombic Pnma	Monoclinic C2/m	Monoclinic C2/m	Monoclinic C2/m
Cell Lattice Dimensions	a = 5.3 b = 9.2 c = 7.3 β = 90°	a = 18.5 b = 17.7 c = 5.3	a = 9.6 b = 18.3 c = 5.3 β = 102°	a = 9.8 b = 18.0 c = 5.3 β = 103°	a = 9.9 b = 18.1 c = 5.3 β = 105°
Occurrence etc	Low grade metamorphism ultrabasic rocks	Metamorphic rocks	Metamorphosed iron formations (ectinite and amphibolites)	In iron formation as metamorphic mineral	Actinolite in iron formations tremolite in metamorphosed calcareous rocks

in detail, very complex; but in essence, the modification can be imagined as a buckling of the double sheet, due to misfits, to form a hollow tube (Deer et al., 1966). This central tube may or may not be filled with electron-opaque material, and the appearance of its image under the electron microscope will be affected accordingly (Yada, 1967). The chemical composition of chrysotile shows comparatively little of the great variability that is found in the amphiboles.

Double chains of silica tetrahedra and metals, octahedrally linked, form the basic structural elements of the amphiboles. The net result is a prominent cleavage parallel to the double chain, and this generally produces an acicular or prismatic habit. Amphiboles show a wide range of chemical composition, reflecting the temperature, pressure, chemistry, and metamorphic history of formation. This wide variation in composition is due to substitution in the basic silica tetrahedra, and the ability of the crystal structure to accommodate a wide variety of different coordinating cations; the result is that all amphiboles, including the asbestos-forming amphiboles, show overlapping composition. Anthophyllite and cummingtonite also have overlapping chemical compositions; as do crocidolite, tremolite-actinolite, and hornblende. Analytical results in the literature often refer to "amphibole fibers", implying that these are understood to be derived from asbestos. However, many nonasbestos amphibole minerals form fibrous or acicular particles when finely divided.

Surface properties. The surface areas of fibrous particles have an important bearing on coagulation and adsorption processes and hence on the ultimate fate of the particulates in the environment. The surface areas of U.I.C.C. (Unio Internationale Contra Cancrum) reference samples have been measured, using both the nitrogen adsorption and the permeability methods (Rendall 1970).

The specific surface of chrysotile is about twice that of the amphibole asbestos minerals. This difference may be due to the greater length-to-width ratio of chrysotile and its porous tubular morphology.

The isoelectric point, the pH at which the net surface charge of a mineral in an aqueous solution is zero, has been measured for chrysotile and cummingtonite. Chrysotile has an isoelectric point of 11.8, and the isoelectric point of cummingtonite is 5.2 - 6.0. Anthophyllite may have a value similar to that of chrysotile, and the other amphibole asbestos minerals may have values similar to those of cummingtonite (Parks 1967). As the pH of the medium falls below the isoelectric point, the surface charge of suspended particles tends to become more positive. Therefore, in a typical drinking water, chrysotile particles should be more positively charged than those of the amphiboles. However, other suspended or dissolved materials can interact with the asbestos minerals and modify their charge.

Morphology. and their morphology is often used for their identification. Fibrils are the individual tubes of single crystals that bundle together to produce a fiber . Chrysotile fibers are usually curved and occur in open bundles, splitting into smaller individual fibrils. Therefore it is often difficult to carry out repeatable size measurements. The fibrils have various diameters, but the average outer diameter is about 200 A. Typical electron micrographs of chrysotile show cylinders, tube-in-tube, and cone-in-cone forms (Whittaker and Zussman 1971). Quite frequently there is a median stripe of greater or less electron density, suggesting that there is a difference in the core compared to the sides of the fiber. A tubular structure, either void or filled with an electron-dense substance, is the simplest explanation of this observation (Yada 1967; Whittaker 1966; Kehieker et al., 1967; Whittaker and Zussman 1971; House 1967).

On the other hand, amphiboles are usually straight and show good cleavage edges parallel to the fiber length and often a second cleavage transverse to the length. The fiber width of UICC samples of anthophyllite often exceeds the fiber width of amosite, which, in turn, exceeds the average width of crocidolite. The approximate minimum fiber widths are: anthophyllite - 2500 A, "amosite" - 1500 A, and crocidolite - 600 A (Timbrell et al., 1970).

Fiber length distributions have been determined for the U.I.C.C. reference samples (Rendall 1970); laboratory experiments have shown that observed distributions depend to some extent on the method used to disperse the fibers for measurement (Timbrell and Rendall 1972). Table IV-4 shows the distribution of fiber lengths by number in U.I.C.C. reference samples, as determined from the analysis of Rendall (1970). When this distribution is compared to milled dusts, the U.I.C.C. reference is seen to approach the lower limit of industrial dust in the case of "amosite", and to approach the upper limit of industrial dust in the case of chrysotile (Rendall 1970). Both in water and in air, the longer fibers (>2 µm) are very rare in the general environment compared to occupational settings.

Solubility. Acid dissolution of asbestos minerals has often been studied in order to test resistance to corrosion (Cotterell and Holt, 1972; Speil and Leineweber, 1969; Choi and Smith, 1971). In general, the resistance to acid solution is chrysotile<"amosite"<actinolite<crocidolite<anthophyllite<tremolite.

Table IV-4. Length Distribution of U.I.C.C. Reference Samples
(after Rendall, 1970)

Mineral Type	% by Number of length greater than (μm):								
	0.2	0.5	1.0	2.0	5.0	10	25	50	100
Amosite	99.9	76.9	65.9	20.3	5.69	1.29	0.21	0.05	0.02
Anthophyllite	99.9	73.2	45.5	22.9	4.79	1.29	0.14		
Crocidolite	100	71.6	35.8	13.3	3.01	0.68	0.08	0.01	0.01
Chrysotile A (Rhodesian)	99.9	79.1	44.6	21.5	6.09	1.26	0.77	0.15	
Chrysotile B (Canadian)	100	69.4	36.0	16.2	3.00	1.24	0.31	0.07	

Occurrence of Asbestos and Fibrous Minerals

Fibrous minerals must be considered to be common wherever unconsolidated sediment occurs because many of the common rock forming minerals can have an acicular morphology (Crailey, et al., 1969). The wide variation in composition, and the crystallographic similarities, as well as the ubiquitous occurrence of fibrous or acicular minerals results in a most difficult analytical problem for envionmental samples. Most analytical studies consider only minerals of the asbestos group.

Asbestos minerals generally occur in metamorphic retrograde deposits. Chrysotile occurs in low grade metamorphic deposits, and the amphiboles occur in slightly higher grade metamorphic deposits. Metasomatism may be a key process in the formation of the asbestos minerals.

Deposits of commercial grade asbestos are mined in Canada and the U.S.A. Chrysotile accounts for 95% of the world production with "amosite" and crocidolite accounting for most of the remainder. The largest known deposits of chrysotile in the world are found in a belt 125 km by 16 km between Danville and Chaudiere, Quebec. Other Canadian deposits are found in Northern Ontario (Matachewan), in Northern British Columbia (Cassiar), and Newfoundland (Baie Verte). In the U.S.A., extensive deposits are found in California, Vermont, Arizona, and North Carolina. The North Carolina deposits are amphiboles, whereas all the other deposits consist of chrysotile. Additional deposits, presently unexploited, are found in other states, notably Montana and Wyoming.

Uses and Redistribution Sources

There are over 2000 recorded uses of asbestos minerals in the U.S.A. (May and Lewis). From this one can conclude that there will be a correlation between population and industrial activity and the concentration of asbestos in the environment. Higher concentrations of asbestos fibers are commonly found in the air and waters around metropolitan areas (Cunningham and Pontefract 1971, Kay 1973).

The following tabulation of uses of asbestos fibers, consisting almost entirely of chrysotile, is of the U.S.A. in 1968 (May and Lewis 1970).

Construction:		88%
Cement products	69%	
floor tile	10	
paper products	7	
paint and caulking	2	
Transportation		3%
Textiles		2%
Plastics		1%
Other		6%

Redistribtion of asbestos minerals to the environment depends on the extent to which the material is conserved or sequestered in the processes of mining, manufacturing and consumption. Emission factors, expressing the fractional loss of asbestos to the environment (on a mass to mass basis) from the various processes and uses have been estimated in two different studies (Davis 1970, Environment Canada 1973), and are shown in Table IV-5.

Table IV-5. Emission factors for asbestos (chrysotile) for the U.S.A. (Davis 1970 and Environment Canada (1973).

Emission factor=mass of asbestos (g) emitted to environment per unit mass (kg) of asbestos in use

	Emission Factor (g/kg)	
	Canada	U.S.A.
Production		
Asbestos mining	4.0	4.5
Asbestos milling	6.0	42
Manufacturing		
Cement Products	0.075	0.5
Floor Tile	0.075	0.5
Paving	0.075	--
Coatings/caulking	0.075	0.5
Insulation	0.15	--
Friction materials	0.45	1.0
Plastics	0.075	--
Textiles	0.15	1.0
Paper	0.075	0.5
Miscellaneous	.075	0.5
Consumption		
Construction Industry	0.075	--
Sprayed Insulation	5.0	5.0
Brake linings		
Installation	5.0	5.0
Wear	20	5.0

When considering redistribution of asbestos in the environment, it is important to distinguish between the mass of material involved and-a more pertinent consideration - the number of fibers of specified dimensions. Note that the production and use estimates, modified by the emission factors, give estimates of emission on a mass basis that can not be directly related to numbers of fibers of particular size ranges. (Size and number are discussed in the following sections on health effects.) Asbestos fibers occur in bundles, individual fibers, and fibrils, and in varying size ranges in the environment. It is not possible in a general sense to relate mass to number.

The natural background concentrations of asbestos fibers in water and in air are not well known. Some minimum concentrations from remote areas suggest that concentrations of 10^4 to 10^6 fibers per liter represent approximate background figures for water, and an ambient air concentration of about 0.01 fiber/cc is an approximate background estimate for air. It may well be that these figures represent a lower limit of detection in a modern analytical laboratory surrounded by materials containing asbestos. In regions that are remote from industrial and populated areas, the median lengths of fibers found in water are generally less than those found in air. Median fiber lengths of < 1 μm are typical, and fibers greater than 2 μm are very uncommon in such cases (Durham and Pang, 1976; Kay 1973). There are reports that asbestos fibers reach the environment by natural weathering (DuBois et al. 1971), but this hypothesis has not been substantiated for areas where asbestos minerals occur naturally, and that have not been disturbed by man.

Asbestos cement pipe is used in some localities to transport drinking water and there has been some concern as to the release of fibers from these pipes (Wright, 1974). Studies have been carried out on the solubility of concrete and cement (Flentje and Schweitzer, 1955;Kristiansen, 1974). In general, almost all soft waters (alkalinity and hardness about 10^{-4} equivalents (mol/l) should dissolve calcium carbonate in the pipe, whereas hard waters typically would not do so. In addition to solubility studies, fiber analyses are being carried out in a pipe-loop laboratory as well as in asbestos cement pipe in soft water areas (EPA, 1975). Results are very tentative at present, but they suggest that some fibers are emitted in corrosive waters.

Exposure of asbestos and other fibrous particle-forming minerals to redistribution may result from the presence of the material in a gangue. The best documented case of this kind of emission is that of the release of fibrous cummingtonite from taconite ore tailings into the Western arm of Lake Superior (Cook et al., 1974). This amphibole occurs together with quartz, tremolite-actinolite, and other minor minerals in both fibrous and non-fibrous forms. A tabulation of the approximate emission rates of fibrous cummingtonite and associated materials gives:

Total emissions	70,000 metric tons/day
Cummingtonite-grunerite concentration	40 wt. %
Fiber concentration, emissions	9x10^{9} fibers/g dry wt. 0.006 g of fiber/g dry wt.
Annual emissions	
total cummingtonite-grunerite	10 million metric tons
no of fibers	2.3x10^{23}
weight of fibers	150,000 metric tons

It is interesting to note that the consumption of asbestos in the U.S.A. in 1968 was about 840,000 metric tons (May and Lewis, 1970) or about 5.5 times the fiber emissions into Lake Superior. If we assume that the average emission factor for asbestos is between 0.08 and 0.15 g/kg, (Table IV-5), the total emissions of asbestos (chrysotile) in the U.S.A. can be estimated to be about equal to the fibrous emission into Western Lake Superior cited above, on a mass basis.

The ultimate fate of the fibrous cummingtonite-grunerite is not well documented. The Western Arm of Lake Superior has water concentrations of cummingtonite-grunerite fibers generally >10 million/liter. The Central and Eastern portions of Lake Superior have concentrations of 2-3 million fibers/liter with nearshore areas being somewhat higher (Kramer 1976). In comparison, a number of inflowing streams have concentrations of 1 to 2 million fibers/liter. If the lake were homogeneously mixed, and no fibers were transported out of the lake, one would expect between 3 million and 5 million fibers/liter from measurements of the emission. From these calculations, one may estimate that 50% or more of the fibers are removed from suspension in the water of Lake Superior, presumably by sedimentation.

Topics for Research in Mineralogy

One of the more important questions is what differences if any, exist between fibers derived from asbestos and those that arise from single crystals or cleavages of single crystals. Furthermore, it is important to be able to develop analytical methods to define these differences, and to relate these differences to health effects. In a recent summary of the mineralogical aspects of this research, Zoltai (1978) concludes that 1) "the mechanism responsible for the development of asbestiform habit may be related to unique surface structures and properties of individual fibers that make up a bundle, 2) electron microscope studies of "amosite" asbestos fibers reveal the presence of narrow bands of polysynthetic twinning and of triple chains interlayered with the usual double chain structure of the amphiboles, 3) most of the natural asbestos ("amosite") fibers have apparently orthorhombic optical properties at a scale of several microns, and 4) some natural asbestos contains adsorbed metals and compounds". Reasons for the development of fibrous habit are not known. Several workers have shown details of variations in structure in asbestos minerals, and these variations must be compared with small scale cleavage fibers from non-asbestos deposits (Chisholm 1973, Hartman 1963, Hutchinson *et al.*, 1975, Ruud *et al* 1976).

ASBESTOS FIBER SAMPLING AND ANAYLSIS

Introduction

A variety of sampling and analysis techniques have been used to identify fibrous or acicular particulates of asbestos minerals and determine their concentrations in air, water, mineral samples and biological tissue. These include optical and electron microscopy, X-ray diffraction, and differential thermal analysis. Identification of fibers of asbestos origin and their estimation in water and tissue samples is difficult for a variety of reasons:

1) Asbestos mineral fibers in water are generally present in low mass concentrations even though the number density of fibers may be high.
2) Many analytical methods can not distinguish between fibers of asbestos origin and particles of other non-asbestos and non-fibrous minerals.
3) Mineral fibers present in water samples are generally smaller than can be resolved with the optical microscope, so an electron microscope must be used.

Methods of estimating mineral fibers in water, and their limitations, are discussed below.

Optical Microscopy

Occupational exposure to asbestos fibers in air is monitored, in the USA, by collecting the fibers on membrane filters and subsequently counting them by phase contrast optical microscopy. For optical microscopy, asbestos fibers are defined as those particles of length greater than 5 µm and a length-to-diameter ratio of 3-to-1 or greater. This method may not be specific for asbestos mineral fibers (by fiber definition), nor can it detect fibers less than about 0.5 µm in diameter.

Petrographic microscopic techniques may be used to identify asbestos mineral fibers greater than approximately 0.5 um in diameter. With the polarizing microscope, various optical crystallographic measurements such as refractive index, extinction angles and sign of elongation may be made and compared with data reported for standard asbestos mineral reference samples.

Dispersion staining with polarized light has been reported as a method for identifying asbestos mineral fibers (Julian and McCrone 1970). Dispersion staining colors may vary depending on the geographic area in which the asbestos was mined. Fibers less than 0.5 µm in diameter can not be identified by this method.

Asbestos fibers present in water and tissue samples are generally too small in diameter for analysis by any of the above optical microscopic methods.

Electron Microscopy

Both transmission and scanning electron microscopy have been used for mineral fiber identification and estimation. In addition to morphological observation, selected area electron diffraction and microchemical analysis may be used to identify fibers.

In addition to their superior resolving power, most modern transmission electron microscopes can be used to observe electron diffraction patterns. Crystalline materials diffract electrons in regular patterns that are indicative of their crystal structures. Visual observation of single fiber (single crystal) electron diffraction patterns may be used to differentiate chrysotile fibers from amphibole fibers (Langer *et al.* 1974, Timbrell 1970, Clark and Ruud 1975). The hollow "central core" of chrysotile fibers may also aid in fiber identification but may disappear with the beam damage (Langer *et al.* 1974). Other fibrous minerals may have hollow cores.

The amphibole mineral fibers are generally straighter in appearance than those from chrysotile. Selected area diffraction patterns for the amphibole asbestos minerals may be similar in appearance; therefore, casual visual observation of these patterns is sufficient only for classification of the fiber as being "an amphibole asbestos" (Langer *et al.* 1974, Cook *et al.* 1974, Clark and Ruud 1975). Electron diffraction patterns of asbestos amphibole minerals are sometimes streaked perpendicular to the fiber length. It is then necessary to study the intensity distribution of the spots within the layer lines to distinguish them from chrysotile.

In addition to visual observation of electron diffraction patterns for fiber identification, photographs can be made of the diffraction patterns and crystal "d" spacings measured from the photographic plates and calculated using the instrument camera constant (Timbrell 1970). Both "spot" and polycrystalline patterns may be measured, however the precision of measurement is not nearly that obtainable with X-ray diffraction techniques. It must be born in mind also that intensities are not the same as observed for X-ray powder patterns and additional reflections may be present.

Although some laboratories are confident of their ability to perform positive fiber identification by electron microscopy with electron diffraction, the method is empirical and has not been rigorously tested. The possibility still remains that other non-asbestos mineral particles may sometimes give diffraction patterns characteristic of chrysotile or the fibrous amphiboles in certain orientations in the electron beam.

Electron beam microchemical analysis may sometimes be used to distinguish microscopic minerals from other fibrous particles (Rubin and Magiore 1974, Ferrell *et al.* 1975, Maggiore and Rubin 1973, Langer *et al.* 1975). The most common system presently in use is the energy dispersive X-ray detector in combination with a scanning or transmission electron microscope. X-ray wavelength dispersive analyzers and the conventional electron microprobe have been used; however, their routine application is limited due to data acquisition times, small particle size, as well as considerations discussed below.

Electron beam X-ray microanalysis coupled only with morphology has often been cited as a method for identifying micro fibers (Rubin and Maggiore 1974, Ferrell *et al.* 1975). However, many scientists have criticized this technique as having several major faults (Ruud *et al.* 1976). First, the elemental composition of amphibole minerals is variable, and seldom ideally stoichiometric. Furthermore many non-amphibole minerals are similar in composition to the asbestos amphiboles and sometimes produce microscopic fibers. Second, the chemistry of the environment, e.g. high ion water, can change the actual or apparent composition of the fiber by ion exchange or surface coating. Third, the variables affecting quantitative microanalysis are such that a plus or minus 10% elemental variance is not uncommon from a single prepared standard sample (Ferrell *et al.* 1975). Electron microscopy with microchemical analysis for fiber identification is reliable only when combined with considerable knowledge of the mineralogy of the fiber source. Such knowledge is generally lacking with water samples.

As has already been discussed, possession of proper elemental intensities by energy dispersive X-ray analysis is generally not sufficient for positive identification of fibers. For example, chrysotile, anthophyllite and fibrous talc, which have similar elemental compositions, may be difficult to differentiate (Rubin and Maggiore 1974, Ruud *et al.* 1976). However, these materials may easily be distinguished using selected area electron diffraction (Ruud *et al.* 1976, Langer *et al.* 1975). Transmission electron microscopes equipped with an energy dispersive X-ray detector are now available that allow simultaneous observation of morphology, crystal structure and elemental composition. These microscope systems have been used to study fibers of known asbestos origin as well as environmental and material samples (Cook *et al.* 1974, Dement *et al.* 1975). The probability of positive fiber identification (including amphibole minerals) is greatly enhanced with these techniques.

Identification and counting of fibrous or acicular particles in environmental and tissue samples has been accomplished by electron microscopy.
Langer *et al.* (1975) have reported a qualitative method for preparing tissue samples for electron microscopy. This method involves first dissolving the tissue in a 40% solution of potassium hydroxide followed by centrifuging. After centrifuging, the residue is dispersed in distilled water and a drop pipetted onto Formvar-coated 200 mesh electron microscope grids. Similar techniques have been used by Pontefract and Cunningham (1973).

Concentrations of fibrous or acicular particulates in environmental samples and biological tissue are usually expressed as fibers per unit volume or weight of sample (fibers/m^3,fibers/liter, fibers/gm dry lung, etc.). These concentrations are determined by counting fibrous or acicular particles within calibrated areas on the electron microscope viewing screen or counting fibers in photographs. Fiber concentrations in water samples determined by laboratories using the same mounting techniques have been reported to vary by a factor of 2 to 3 (Cook *et al.* 1974). Much larger variations have been reported between laboratories using different techniques (Brown *et al.* 1976).

The mass of chrysotile concentrations in environmental samples has also been determined using electron microscopy. This is accomplished by measuring the length and diameter (volume) of each fiber and calculating mass using the appropriate density (Selikoff *et al.* 1972). The accuracy of this technique has not been studied in detail.

X-Ray Diffraction

X-ray powder diffractometry is one of the standard mineralogical techniques used in the analysis of solid crystalline phases. X-ray diffraction has been widely used for identification and quantitation of fibers in bulk materials such as talc (Stanley and Norwood 1973, Rohl and Langer 1974) and other industrial materials (Crable and Knott 1966a, 1966b, [illegible] and Lynch 1970). X-ray diffraction has also been used to [illegible] amphibole asbestos contamination of water samples (Cook *et al.* 1974). However application of X-ray diffraction to routine asbestos fiber analysis of environmental samples has been limited. Birks *et al.* (1975) have reported a feasibility study concerning quantitative analysis of airborne asbestos. This technique however is still in the research phase and has not been developed to the reliability of a general analytical method.

It must be recognized that with the exception of the technique described above, x-ray diffraction methods are not capable of differentiating between asbestos mineral fibers and their non-fibrous mineral counterparts. This fact, combined with the relatively low sensitivity suggests that analysis of environmental samples by x-ray diffraction should be confirmed by electron microscopy.

Differential Thermal Analysis

Differential thermal analysis has been used to determine asbestos mineral fiber levels in talc samples (Schelz 1974).

It has not been used for environmental samples because it has low sensitivity. Moreover, it is not capable of differentiating between asbestos fibers and their non-fibrous mineralogical polymorphs.

BIOLOGICAL EFFECTS OF ASBESTOS MINERALS

Epidemiological Findings

Numerous epidemiological studies have shown that occupational exposure to asbestos dust can lead to asbestosis (characterized primarily by pulmonary fibrosis); the formation of pleural plaques; a greatly increased risk of bronchogenic carcinoma; pleural mesothelioma; and peritoneal mesothelioma (Selikoff *et al.*, 1973; Newhouse, *et al.*, 1972; Elmes and Simpson 1971; Selikoff and Churg 1965; Rogovski *et al.*, 1973, and Lee 1974). There is also evidence that the elevated risk of bronchogenic carcinoma as a result of occupational exposure to asbestos dust is largely (though perhaps not entirely) confined to cigarette smokers (Hammond *et al.*, 1975). Different types of asbestos may vary in their potency in relation to the effects mentioned above; but this has been difficult to evaluate owing to lack of precise information on degree of exposure and various other problems.

Although there is little evidence concerning the possible effects of non-occupational exposure, some cases of mesothelioma have been found among persons working in or living near shipyards and the wives of asbestos workers who handled the dusty clothes of their husbands (Newhouse and Thompson 1965; Wagner *et al.*, 1960; Anderson *et al.*, 1976).

There has been much discussion of the possibility that the effects of inhaling asbestos mineral dust vary greatly with the length of the particles. However, no epidemiological evidence is available on this matter. The dust to which industrial workers are exposed typically consists of fibers varying in length from very long to extremely short. The presence of fibers sufficiently large to be seen under a light microscope is invariably accompanied by the presence of large numbers of far smaller fibers.

It should be noted that all of the epidemiological evidence noted above has come from studies of people who have been exposed to dust from types of asbestos minerals that have been used commercially. There is no direct epidemiological evidence on the effects of various other types of fibrous minerals, some of which may perhaps find their way into drinking water.

The fact that exposure to air heavily polluted with asbestos mineral fibers often leads to the diseases mentioned above does not necessarily indicate that drinking water contaminated with an equally large number of such fibers may lead to the same diseases or perhaps some other diseases. (Site of cancer can vary depending upon the way in which individuals are subjected to a carcinogenic agent, for example: skin exposure, inhalation or ingestion.) However, the hypothesis is tenable to the degree that it cannot be ruled out of consideration without evidence to the contrary.

It is of importance to note that workers exposed to air containing large numbers of asbestos minerals fibers inevitably ingest such fibers. Many of the fibers that are inhaled are later propelled upward from the lungs and trachea, enter the mouth, and are then swallowed. Thus fibers are brought into direct surface contact with the epithelial lining of the buccal cavity, esophagus, stomach and intestines. For this reason, it is pertinent to inquire whether death rates from cancer arising in these tissues are higher among asbestos workers than in the general population, age, sex and calendar-years of exposure being taken into consideration. This has been investigated by Selikoff, Hammond, Seidman, Churg and their associates. The data shown on Table IV-6 were provided by these investigators. It shows, for each of two groups of workers, the observed and expected number of deaths from cancer of sites directly exposed to asbestos fibers by way of ingestion: buccal cavity and pharynx, esophagus, stomach, colon and rectum. The two groups are described below.

Group #1. The entire membership of the insulation workers union in the United States and Canada was registered as of January 1, 1967 (17,800 men). Many characteristics of the men were recorded including date of birth and onset of work with asbestos. These subjects have been traced through December 31, 1976.

Group #2. This group consisted of the entire workforce (1941-45) of a factory in an eastern U.S. city that manufactured amosite asbestos insulation materials (including insulating block and pipe covering, and asbestos mattresses), for use by insulation workers in the construction industry, especially in ship construction and repair. The plant opened its doors in June 1941 and remained in business until November 1954. Very few of these production workers studied had had prior occupational exposure to asbestos. Between 1941 and the end of 1945 a total of 933 men were hired; of these, some worked for as little as one day, while others worked until 1954 when the plant closed. Of the 933 men, 881 (94.4%) were traced through December 31,1973 and the remaining 52 were traced for a part of the period but later lost to follow-up.

For both groups, "observed", as shown in Table IV-6, means the number of men who were found to have died of the indicated cause during the period of observation. "Expected" numbers of deaths are based upon age-specific death rates for white males in the United States during each year of observation.

Group #1 was by far the larger of the two groups, and in consequence the figures are more stable statistically. In this group, the observed number of deaths was substantially higher than the expected number for cancer of each of the four sites. The findings were similar for Group #2 except in respect to cancer of the esophagus (zero observed vs 1.36 expected deaths).

In each of the two groups, the excess of observed over expected number of deaths (for all four sites of cancer combined) was highly significant statistically. It may be argued whether U.S. white males are an appropriate control group in relation to Group #2 (New Jersey "amosite" workers). Therefore the expected numbers for Group #2 were re-computed based upon New Jersey mortality data. These expected numbers were a little higher than those shown in Table IV-6.

Similar findings in respect to gastro-intestinal cancer have been reported by Mancuso (1965) and by Elmes and Simpson(1971). This, taken together with the data presented in Table IV-6, strongly suggests that ingestion of asbestos mineral fibers can result in an increased risk of cancer of several sites. If so, the degree of risk is probably highly dependent upon the number of fibers ingested and the duration of exposure. It may also depend upon the type and size of the fibers as well as upon other agents to which the individuals have been exposed. For example, both smoking and alcohol increase the risk of cancer of the buccal cavity; and it is possible that exposure of the buccal mucosa to asbestos mineral fibers simply increases the risk of cancer occurring among persons also exposed to one or both of these other two factors.

As described elsewhere in this report, asbestos mineral fibers - varying in number from trace amounts to hundreds of thousands per liter - are present in drinking water in various locations in the United States. The situation in Duluth, Minnesota (as described elsewhere) is of special interest from an epidemiological point of view.

Studies of time trends in cancer death rates and incidence rates in Duluth (as compared with other cities) do not show any increases that could reasonably be attributed to the mineral fiber pollution of the drinking water of that city (Mason et al., 1974; Levy, et al., 1976).

Among persons occupationally exposed to asbestos dust, the increases in death rates from cancer of the stomach, colon and rectum do not reach measurable proportions until many years after onset of exposure. The lapse of time since Duluth water was first heavily polluted with mineral fibers is not yet sufficient to have produced a significant increase in death rates from these cancers, even if it be assumed that the risk for residents of

Duluth is as great as the risk for workers occupationally exposed to asbestos dust. If the risk is not so great - but still of important proportions - it will probably become apparent within another five, ten, or fifteen years from now. An even lower risk might not become apparent for a much longer period of time - and then it would be difficult if not impossible to pinpoint the cause.

TABLE IV-8. Observed vs expected number of deaths from cancer of several sites among two groups of workers occupationally exposed to asbestos dust. Data provided by Selikoff, Hammond, Seidman and Churg.

	Group #1. Asbestos Insulation Workers (U.S.A. and Canada)			Group #2. "Amosite" Asbestos Factory Workers (New Jersey)		
Site of Cancer	Obs. Deaths	Exp. Deaths	Ratio	Obs. Deaths	Exp. Deaths	Ratio
Buccal and pharynx	15	7.87	1.91	6	2.04	2.94
Esophagus	14	5.38	2.60	0	1.36	----
Total of above	29	13.25	2.19	6	3.40	1.76
Stomach	18	11.25	1.60	10	4.89	2.04
Colon-Rectum	47	28.64	1.64	16	7.65	2.09
Total of above	94	53.12	1.77	32	15.94	2.01

Experimental Studies

As discussed above, epidemiological studies of the population exposed have failed to detect any increase in gastrointestinal cancer that might be ascribed to the asbestos mineral fibers contained in some drinking water. But, since these results do not indicate conclusively that ingestion of water containing such fibers is without risk, and because these substances are widely distributed, it is important also to consider experimental evidence.

An Advisory Committee on Asbestos Cancers to the Director of the International Agency for Research on Cancer, meeting in 1972, considered whether there was evidence of an increased risk of cancer resulting from asbestos minerals present in water, beverages, food, or in the fluids used for the administration of drugs. They concluded that "such evidence as there is does not indicate any risk" (IARC 1973). The group recommended that the effect of long-term ingestion of fibers of various sizes, shapes and chemical compositions should be studied. The advisory committee's conclusion can hardly be taken as a categorical denial of risk, but rather reflected the inconclusive character of the evidence then available.

Published results attest to the difficulties experienced by many investigators in studying, by means of experiments with animals, the biological effects and behavior of inorganic fibrous materials in general, and asbestos mineral fibers in particular. Several different approaches have been taken to the design of such experiments, including administration of different types and preparations of asbestos minerals and glass fibers to several species of experimental animals, by ingestion (feeding), inhalation, surgical implantation and injection. Experiments have also been conducted to test the response to fibers of cell cultures *in vitro*. The results of some representati experiments of these kinds are reviewed below.

Westlake *et al.* (1965, 1974) fed chrysotile to rats and noted the presence of fibers of the material in many sites in the colonic epithelium and lamina propria; and Webster (1974), after feeding crocidolite to baboons, showed that small numbers of fibers appeared in the ashed tissue of the gut wall. Gross *et al.* (1974) reported no such penetration. Bolton and Davis (1976), reporting on the short term effects of chronic ingestion of asbestos minerals by rats, found no sign of cell penetration by fibers or damage to the gut mucosa. They concluded that penetration of the gastrointestinal wall, if it occurred, must have been on a very small scale.

Pontefract and Cunningham (1973) reported that chrysotile, administered to rats intragastrically by direct injection, was later found to be distributed to other organs, and suggested that the material penetrated the digestive tract. Gross (1974) questioned this interpretation of the study on the grounds that the method of administration was likely to have produced contamination of other organs by leakage. Cunningham and Pontefract (1974) reported the appearance of asbestos mineral fibers in fetal organs after injection of asbestos into the femoral veins of pregnant Wistar rats. While none of these papers demonstrated transport of fibers across tissue boundaries some included the suggestion that the reported deposition of the material in other organs implies that transport occurred. Reports such as these have, without resolving the matter, lent interest to the question whether or not human ingestion of asbestos may be accompanied by similar effects.

Experimental investigations with animals of the toxic effects of asbestos mineral fibers have not yet led to the development of an experimental model system that reproduces the putative effects of ingestion of such fibers by man. Nevertheless, various animal studies on the toxicity of asbestos have been reported, and some large-scale feeding experiments are now in progress.

In the experimental studies by Wagner *et al.* (1974), using the U.I.C.C. reference samples, no association was found between exposure to asbestos by inhalation and excess gastrointestinal cancers. Thus, this animal study did not duplicate the positive epidemiological findings, previously discussed. The possible effects of ingested asbestos also were studied experimentally. Several animal feeding studies with asbestos are reported to have shown no increase in cancer with this route of exposure (Bonser and Clayson, 1967; Smith 1973; and Gross *et al.*, 1974). The asbestos minerals tested included crocidolite, chrysotile and amosite. However, none of these studies would be considered adequate under modern constructs of chronic toxicity testing (National Academy of Sciences, 1975; Sontag *et al.*, 1976), and so the possibility of false negatives remains.

More recently, Gibel *et al.* (1976) fed filter material, containing asbestos, (20 mg/day) to 50 Wistar rats. This material was composed of asbestos (chrysotile, 53%), sulfated cellulose and a condensation resin. The authors reported 12 malignant tumors in the group that was fed the filter material, 2 malignant tumors in a control group, and 3 in a similar group of rats that were fed the same amount of a standard grade of talc. The 12 malignant tumors appearing in the group receiving the filter material comprised 1 lung carcinoma, 3 reticular cell sarcomas, 4 kidney carcinomas and 4 liver carcinomas. In the control animals and the talc-fed animals the malignant tumors were all liver carcinomas. The average survival times were 441 days in the filter-fed group, 702 days in the controls and 649 days in the talc-fed group. The presence of other substances besides asbestos in the filter material, and the manner of reporting the malignant tumor counts suggests that further studies are needed, with asbestos alone, before firm conclusions can be drawn from the results of this study.

A fundamental difficulty associated with these studies is that they seek to duplicate an effect in man by means of animal models that have neither been validated for the route of administration nor for the materials of interest. Negative results may be interpreted as indicating either the absence of the effect under investigation or, simply, as further proof that the model was not valid to start with. At present, the existing animal data base leaves the matter unresolved.

Results obtained from some experiments with animals by direct application of fibrous materials, and with *in vitro* systems, have been adduced to support the view that ingestion of drinking water containing mineral fibers should not be expected to lead to any observed increase in gastrointestinal cancer in the general population. These experiments are interpreted to show the dependence of biological effect on the size of fibers. In this approach, large quantities of mineral fibers, of various size distributions, are injected into the pleural or peritoneal cavity. Stanton *et al.* (1969) wrap a plaque of fibrous material around the lung in their animal model studies. This discussion will be limited to the results reported with respect to a carcinogenic response.

The results obtained from these experiments, in which the material under investigation is deposited or applied directly, are no better than the statistics of their individual design nor the method of fiber preparation and determination of the fiber size distribution. They may be summarized as follows:

1. Tumor induction is related to fiber size, shape and durability, and not to the source of the fiber or its chemical composition (Stanton and Wrench, 1972; Stanton, 1973; Stanton *et al.* in press; and Pott and Fredricks, 1972; Pott *et al.*, 1976).

2. In cell cultures, particles less than 20 μm in length do not induce growth of fibroblasts (Maroudas *et al.*, 1973).

3. Chrysotile containing particles less than 5 μm in length did not elicit a carcinogenic response (Smith, 1974; Smith *et al.*, 1972).

4. Defining a "significant" asbestos fiber as one less than 0.5 μm in diameter and greater than 10 μm in length, there was good agreement between the number of these "significant" fibers in an asbestos sample and its degree of carcinogenicity (Wagner *et al.*, 1973).

5. Man-made mineral fibers less than 1.5 µm in diameter and greater than 8 µm in length have the highest probability of inducing a biological response. The response appears to increase with increasing fiber length at fiber diameters less than 1.5 µm (Stanton _et al_., in press).

In contrast to the results summarized above, two other investigations appear to show a different dependence of biological effect on fiber length. In one of these, Pott _et al_. (1972) injected 100 mg of two different preparations of chrysotile intraperitoneally, one sample contained 95% of particles less than 5 µm in length and the other (a milled sample) contained 99% less than 3 µm in length. Both groups gave approximately the same tumor incidence, although the latency period was greater in the latter group. The data subsequently were reported in greater detail by Pott _et al_., (1974). In this, for the unmilled chrysotile, 78.7% of the fibers were less than 2 µm in length and 93.9% were less than 5 microns in length; while for the milled sample, 97.4% were less than 2 µm in length and 99.8% were less than 5 µm in length. For the unmilled chrysotile sample, the elapsed time to appearance of first tumor was 370 days with a tumor incidence of 37.5%; while for the milled chrysotile sample the time to appearance of first tumor was 400 days, with a tumor incidence of 30%. This study would indicate that both short and long fibers may be carcinogenic, though the milled sample seems somewhat less active than the unmilled one. But in the absence of information about the distribution of fiber diameters and the number per unit weight it is impossible to assign activity to a particular size class with certainty.

Another possible exception is contained in a paper by Wagner _et al_. (1976), describing experiments with glass fibers. In these experiments two different preparations of glass fibers were injected intrapleurally into experimental animals. The characteristics of the fiber preparations and the results are summarized below.

Characteristics of Glass Fibers

	Finer	Coarser
diameter <0.5µm	99%	--
diameter <1µm	--	17%
median diameter (µm)	0.12	1.8
length >20µm	2%	--
length >50µm	--	10%
median length (µm)	1.7	22
Results		
No. with mesothelioma	4/32	0/32
Absence of hyperplasia in mesothelial cells	1/32	12/32

The same weight (20 mg.) of each material was used per animal, and there were about 1,000 times more fibers in the finer glass than in the coarser glass (Wagner *et al.*, 1976). The results indicate, therefore, that the finer sample contained more "active" fibers than did the coarser.

On the basis of the animal experiments noted above it is possible to infer, that in these experimental systems, short fibers may be biologically unreactive, or less reactive, than longer ones. Since the majority of the fibers that are found in water are short (<5μm.), these may be similarly unreactive, and this may account for failure to detect any increased incidence of various gastrointestinal cancers related to ingestion of mineral fibers in water.

Another hypothesis can be offered for the inability to detect, in the general population, any increase in various gastrointestinal cancers attributable to ingestion of asbestos mineral fibers in water. Assuming that the heavily exposed asbestos workers (who showed an excess gastrointestinal cancer rate) swallowed a large fraction of the fibers, of all sizes, that they inhaled, the dose so acquired would have been several orders of magnitude greater than that ingested in drinking water by members of the general population. It would therefore be expected that any excess gastrointestinal cancer due to fibers in water would be much smaller in the general population than that found in asbestos workers, and if sufficiently low, might be very difficult to detect against the usual background incidence of cancers of the colon, sigmoid, and rectum and less so for cancers of the esophagus and stomach.

The results of experiments on the chronic toxicity of asbestos mineral fibers fall very far short of rigorously clarifying the risk to health that may be associated with potable water -- either qualitatively or quantitatively. Although no harmful effects of fibrous contamination of drinking water on the present state of public health have been demonstrated, it is possible, as noted in the discussion of the epidemiology, that they may be observed in the future.

The development of gastrointestinal cancers among the heavily exposed asbestos workers has been slow (20 to 30 years or more), and the possibility of long-delayed effects of mineral fiber ingestion through water cannot be ignored. Research on the unresolved problems of the chronic effects of ingesting asbestos mineral fibers therefore merits strong support.

ORGANIC PARTICULATES IN WATER

The organic particulate matter found in natural water systems may be classified as follows:

1. Organic matter associated with soil particles.
2. Organic particles from effluent of sewage treatment facilities and industrial waste disposal facilities.
3. Plant and animal debris.
4. Microorganisms.
5. Organic colloids.

Microorganisms are discussed elsewhere in this report; the other categories are discussed below.

Organic Matter Associated with Soil Particles

The major part of the suspended material in most natural bodies of water is made up of soil particles d[illegible] from the land surface by erosion. The coarser sand and silt fractions consist mainly of inorganic rock and mineral fragments, many of which are, at least partially, coated with organic material. The clay size fraction of most soils consists of clay minerals, metal oxides, hydrous metal oxides and soil organic material (humus) in intimate contact with one another. As Gorbunov, Yerokhina and Schchurina (1971), and Greenland (1971) have shown the clay minerals in the A horizons of most soils are coated with organic matter, forming a so-called "clay-organic complex".

Greenland also found that hydrous oxides provide even more sites for adsorption of organic matter in soils than do micaceous clay minerals. Hydrous oxides also coat surfaces of clay mineral grains. It is therefore to a certain extent artificial to attempt to separate the effects of the organic and inorganic components of the soil particulate matter suspended in water. However, it is likely that in many instances the effect of the organic components is predominant.

In those instances in which the soil organic material (humus) forms coatings on the clay and oxide surfaces of soil particles, reactions between the coated particles and the environment external to the particles will to a large extent be governed by the physical chemical properties of the humus. For example, the ion exchange capacity, and the adsorption properties of the particles will be strongly influenced by the ion exchange capacity and the adsorption capacity of the humus coatings.

Both soluble and insoluble organic compounds are sorbed by humus. Ladd and Butler (1971) have shown that the humic and fulvic acid components of soil humus inhibit proteolytic enzymes by binding them. In addition to enzymes, humus strongly binds some pesticides and other organic compounds that are found in soils and natural waters. This sorption reduces the phytotoxicity of herbicides and often inhibits the degradation of many pesticides (Wershaw and Goldberg, 1972).

In order to understand the interactions of soil particles in natural water systems, it is necessary to consider briefly what is known about the chemical structure and physical-chemical properties of soil humus. Humus has been traditionally fractionated into three compounds: Humic acid, fulvic acid and humin. Humic acid is that component which is soluble in strong base but insoluble in strong acid, fulvic acid is soluble in both acid and base; and humin is insoluble in both acid and base.

Humic acids contain phenolic, quinoid, polycarboxylic aromatic acidic, and aliphatic groups, the aromatic groups predominating (Kononova, 1966; Felbeck, 1965 and 1971; Scheffer and Ulrich, 1960; Steelink, 1963; Flaig, 1970). In addition to benzene carboxylic acid and phenolic groups, some authors have included fatty acid, protein, and polysaccharide groups. It has been assumed that these groups are joined together covalently by linkages such as ether, ester and carbon-carbon to form three-dimensional polymers of high molecular weight (Flaig, 1970).

Several recent studies indicate that this structural interpretation is not correct. Wershaw and Pinckney (1971, 1973a, and 1973b) have shown that the most abundant humic acid fractions form aggregates in solution that are made up of relatively small molecules (molecular weights of several thousand or less). The degree of association of these molecules is a function of pH. Monahan, DeLuca and Wershaw (1972) have found that the degree of aggregation of some of the fractions is also a function of concentration and that at low concentrations the molecules are dissociated. The presence of these aggregates will have a marked effect on the way humic acids interact with pollutants in natural water systems, and this will be discussed later.

Fulvic acids are generally believed to be similar in structure to humic acids but of lower molecular weight. It is apparent, however, that other groups of compounds, such as polysaccharides, proteins and amino-sugars are also included in many fulvic acid preparations (J.A. Leenheer, oral comm. 1974).

Alternating treatment of the humin fraction with stong mineral acids and strong bases generally renders most of the humin soluble in basic solutions (Kononova, 1966). From this

Kononova has concluded that humins are humic acids that are bound by the mineral constituents of soils.

Humic substances have a large capacity for base exchange. Kononova (1966) has reviewed the exchange capacity of several different soils and has reported values from about 200 to over 500 milli-equivalents per 100 grams for humic acids. The ion exchange capacities of humic acids depend on pH and are smaller at low pH values than at high pH values.

There has been a great deal of study lately on trace metals in natural water systems because of the effect that variations in the concentration of those metals can have on plant and animal health. Harding and Brown (1975) have studied the distribution of some selected trace metals in sediments of the Pamlico River Estuary in North Carolina. They found the highest concentrations of trace elements in the mixed clay organic matter sediments in the center of the estuary. These high trace metals concentrations are apparently due to human activity. Concentrations 4 to 1750 times normal background were detected. Their evidence suggests that the trace metals are scavenged from water entering the estuary by the clay and organic matter in the sediments. The suspended clay and organic matter in the water would also be expected to have high trace metal concentrations. Shimp et al (1971) have found that the concentrations of bromine, chromium, copper, lead, and zinc increase with increasing organic carbon concentrations in recent sediments from southern Lake Michigan. They pointed out that: "High concentrations of these elements correlate more closely with the amounts of organic carbon present than with clay-size material, water depth, iron oxide or manganese oxide."

A very significant portion of the cation exchange capacity of most soils and marine sediments is due to organic polyelectrolytes; in soils and sediment of high organic content, most of the exchange capacity can be attributed to organic polyelectrolytes (Schnitzer, 1965, and Rashid, 1969). Schnitzer and Skinner (1965) found that both carboxyl and phenolic hydroxyl ligands react with polyvalent cations. Infrared studies by Rashid (1971) on marine humic acids confirmed the participation of carboxyl groups in exchange reactions with cations, but no evidence for the participation of phenolic hydroxyl groups was found.

Koshy and Ganguly (1969), Malcolm (1969), and Ong *et al.* (1970) have studied the formation of soluble humic acid-metal complexes. They found that the amount of metal ions that is complexed by humic acid increases with increasing pH. Although these authors did not offer an explanation for this behavior, it is reasonable to suppose that with increasing pH more disaggregation of the humic acid takes place, and more ligand sites are exposed for complexing with the metal ions. The metal ions may be bound both by ligands on a single molecule or ligands on more than one molecule. The metal ions may therefore act as bridges between molecules, causing aggregation (Sipos *et al.*, 1972).

In addition to interactions with metals, humic acids and other natural organic polyelectrolytes can interact strongly with other organic components in natural water systems. These interactions can be of two types: incorporation of organic molecules into humic acids during their formation, and reaction of organic compounds with humic acids after their formation.

Humic acids are generally considered to arise from the microbial degradation of organic debris. Martin and his coworkers (1972) have shown that some soil fungi can convert a wide variety of different organic substrates into phenolic and quinoid compounds and aromatic acids, that are then converted into humic acids.

Some studies have also revealed that some humic acid fractions have high free radical content. Steelink and Tollin (1967) and others have found free radicals both in soils and in humic acids isolated from soils. The evidence suggests that the radicals detected are quinoid radicals. Martin has not postulated a mechanism for the conversion of the phenols, quinones, and aromatic acids into humic acids, but it is likely that free radical polymerization reactions play a major role in this conversion.

The free radicals that polymerize into humic acid molecules probably arise either from enzymatic reactions or photolysis. Compounds that are either oxidized or reduced to free radicals may be derived either from plant and animal remains, or from sewage, pesticides, or other pollutants. The conditions for these oxidation-reduction reactions are undoubtedly present in many soil-water systems. Su and Zabik (1972) have studied the photolysis of the miticide m-(N,N-dimethylformamidino) phenyl N-methylcarbamate in water. They have shown that decomposition proceeds through the formation of free radicals. Similarily Mazzocchi and Rao (1972) have found that radical intermediates are formed in the photolysis of the herbicides, monuron and fenuron.

Bordeleau, et al, (1972) have studied the degradation of phenylamide herbicides in soils. They found that azobenzenes and polyaromatic compounds are formed, by a combination of enzymatic and chemical reactions, from the phenylamide herbicides. Chloroaniline groups are formed by acyl-amidase degradation of phenylamide herbicides. These chloroaniline groups are then transformed to stable chloroazobenzene residues by peroxidases. During the enzymatic degradation by peroxidase, free radical intermediates were detected by electron paramagnetic resonance. The free radicals produced in the above reactions could be incorporated into humic acids. The work of Khan and Schnitzer (1972) strongly suggests that different organic molecules are indeed incorporated in humic acid. They have succeeded in releasing a variety of hydrophobic organic compounds from a humic acid after exhaustive methylation with diazomethane. They were able to identify alkanes, fatty acids, dialkyl phthalates and butyl adipate in the mixture of released compounds. These compounds comprised about 1% of the total humic acid weight. Previous extraction with organic solvents had released only a very small amount of the hydrophobic compounds. Khan and Schnitzer have proposed that humic acid has a molecular sieve type of structure that traps insoluble organic compounds. This mechanism implies that humic acid molecules form a three dimensional network with suitable voids in which the hydrophobic molecules are adsorbed. Khan and Schnitzer apparently assume that methylation disrupts this structure and releases the entrapped compounds. The humic acids that were used by Khan and Schnitzer were extracted from soil with sodium hydroxide solution. During this type of extraction disaggregation of the humic acid molecules will take place and most of the molecules trapped in the voids should be released. The compounds that they identified were apparently not released and therefore another mechanism must have been responsible for binding these molecules. The fact that methylation was required for release of the molecules strongly suggests that they were chemically bonded to functional groups in the humic acid polymers and could well have been incorporated into the humic acid polymer when it was formed.

The humic acid polymer, after formation, may participate in reactions which may be conveniently divided into the categories tabulated below. When considering these types of interactions it must be kept in mind that humic acids form molecular aggregates; changes in the degree of aggregation will probably modify these interactions.

Mechanisms of Interaction

Physical Interactions

Adsorption
- a. Hydrogen bonding
- b. Van der Waals bonding

Chemical Interactions
- Chemisorption
- Coordination reactions
- Radical and other chemical reactions

Most organic pesticides are adsorbed by soil organic matter (Wershaw and Goldberg, 1972). A few studies of the mechanism of adsorption of particular pesticides by soil polyelectrolytes have been conducted. Sullivan and Felbeck (1968) and Li and Felbeck (1972) have proposed that the sorption of triazine herbicides is due either to hydrogen bonding or to ionic bonding or to both of these taking place simultaneously. Hsu and Bartha (1976) found that up to 90 percent of the 3,4 dichloraniline (DCA) derived from the biodegradation of phenylamide herbicides is adsorbed so strongly by soil organic matter that it is not extractable by solvent extraction. The bound DCA is susceptible to acid and alkaline hydrolysis. The authors have concluded that this suggests that the nitrogen atom of the DCA is covalently bound to the carbon of a carbonyl group or to a quinoid ring of a humic acid molecule. Much more work is necessary, however, to elucidate the general principles of adsorption of pesticides by soil organic polyelectrolytes.

The evidence cited above for the strong adsorption of many pesticides by soil organics suggests that one would expect these pesticides to be associated with the sediments in rivers and lakes, and indeed this has been found to be the case.

Manigold and Loral, (personal communication 1976) have found in a study of the distribution of pesticides in rivers that the chlorinated hydrocarbon insecticides such as DDT, are concentrated in the suspended sediments and that very little of these materials is found in solution in the water. A similar situation would be expected with the polychlorinated biphenyls and other hydrophobic pollutants in natural waters.

An example of chemisorption by humic acids appears to be found in the work of Perry and Adam (1971). They studied the incorporation of peptides into humic acid by allowing glycylglycine to react with humic acid at pH 8.5. They found that part of the amino nitrogen introduced into humic acid by this reaction could not be removed by hydrolysis with 6N HCL at 110°C for 24 hours. At least part of this unreleased amino nitrogen is probably bound to the humic acid by N-phenyl linkages. Although the mechanism of formation of these bonds has not been elucidated, this example illustrates the reactivity of humic acids and the strengths of the bonds formed. It also suggests that chemisorption accounts for much of the binding of amino acids, peptides, and proteins that has been observed in soils. Anderson (1958) has shown that the hydrolysis products of deoxyribonucleic (DNA) are released from humic acid by perchloric acid hydrolysis; he concluded from this that DNA is present in humic acid. The binding of enzymes to humic acids (Ladd and Butler, 1971; Mato *et al.*, 1971) is probably also due in part to the formation of bonds between amino nitrogen groups and reactive sites on the humic acid molecules. Undoubtedly other types of reactions can also take place, some perhaps involving shared metal cations. A careful study of the interactions of humic acids with enzymes should give a new insight into the biochemical reactions that take place in soil-water systems.

Municipal and Industrial Wastes

Municipal and industrial waste disposal is a major source of organic particulate matter in natural waters that serve as sources for drinking water. The amount of particulate matter added by any particular waste disposal site is highly dependent on the amount of treatment that is given to the wastes at the site. (Litchfield, 1975; Soderquist, 1975; Dove and Gillman, 1975; Jewell et al. 1975; Talbot, 1975; Pico, 1975; Macauley, 1975). Large quantities of organic and inorganic particulate wastes are also added to natural water from urban runoff and from combined sewer overflows (Field and Knowles, 1975). These sources supply very large quantities of pollutants to natural waters during periods of heavy rainfall, so that the amount of pollution is highly variable. This makes it particularly difficult to deal with because it is very hard to predict the quality of the feed water into a drinking water plant that is being supplied with water from a source that is subject to intermittent pollution caused by storm runoff. In combined sanitary storm sewage systems untreated sanitary sewage may be discharged into a receiving stream during periods of high storm sewage flow.

Most of the organic matter supplied to natural waters by sanitary sewage treatment plants and treatment facilities for food processing plants are rapidly degraded in rivers and lakes, as evidenced by their high biological oxygen demands. However, some industrial products are much more persistent. Giger et al. (1974) in their study of the sediments in Lake Zug, Switzerland, found high concentrations of petroleum-derived hydrocarbons in the sediments near densely populated areas. They concluded that biodegradation of these hydrocarbons in the sediments is retarded.

Shelton and Hunter (1974) have studied the occurrence of oil pollutants in sediments. They found that the heavier petroleum fractions are concentrated in sediments. In the event of an oil spill, petroleum will be adsorbed by the sediment in the area of the spill and will be slowly released. As time passes, the petroleum components remaining in the sediment will become heavier and heavier.

The fine grained particulate matter found in rivers and lakes that drain heavily populated areas are generally high in organic carbon and in trace elements. Much of this material is derived from domestic and industrial waste disposal. For example high concentrations of metals are often found in sewage sludge and the organic particulates released by sewage treatment plants (Bruland et al., 1974; Leland, Copenhaver and Wilkes, 1975).

An example of high trace metal concentrations in suspended sediments of a heavily industrialized drainage basin is found in Southern Lake Michigan. Leland, et al. (1973) have studied the distribution of trace metals in the bottom and suspended sediments in Southern Lake Michigan. Their data show markedly higher concentrations of arsenic, bromine, chromium, copper, mercury, lead and zinc"...near the sediment water interface of fine-grained sediments than in underlying sediments of Southern Lake Michigan". They found that with the exception of bromine the trace element concentrations in the suspended sediments were equal to or higher than the concentrations in fine-grained surficial sediments. They concluded from this evidence that the high trace metal concentrations in the sediments are due to high concentrations in the suspended sediments that settle out in the lake. The highest concentrations of trace elements are in the central basin of the lake, where the finest sediments are found. Thus the smallest suspended particles, which do not settle out until they have reached the center of the lake, have the highest concentrations of trace metals. These sediments also have the highest organo-chlorine insecticide concentrations. Leland, Shukla and Shimp (1973) found a positive correlation between the organic carbon concentration, iron oxide concentration, and trace metal concentrations in the sediments.

Since many of the communities around Lake Michigan take their drinking water from the lake, the high trace metal and pesticide concentrations in the suspended sediments could pose a problem if the coagulation and filtration processes used in water treatment are not adequate to remove them from the water.

When organic particulate matter containing high trace metal concentration is introduced into a natural body of water a new set of equilibrium equations will in general be required to represent the system. If the chemical composition of the water is different from that of the solutions in which the particulate matter acquired the trace metals redistribution of metal ions between the water and the sediment phases will take place. This sediment will serve as a source of trace metals in water even after the source of pollution is removed.

Release of metals fixed to the suspended and bottom sediments into a body of water may also take place by decomposition of the organic matter binding the metals in the sediment. DeGroot, DeGoeij and Zegers (1971) have reported observing this phenomenon in the Rhine and Ems Rivers. They found that most of the mercury and other heavy metals transported to the sea by the rivers are fixed to suspended particles of less than 16 micrometers in diameter. The suspended sediments of the Ems River have much lower concentrations of heavy metals than the Rhine River because the drainage basin of the Ems is much less industrialised than the Rhine Basin. The metal ions remain fixed to the suspended particles in both of the rivers until the chemical composition of the river water is changed by mixing with sea water. Downstream from the freshwater tidal zone of each of the rivers mercury, zinc, lead, chromium, arsenic, cobalt and iron are lost from the sediments. The authors have attributed this to decomposition of the organic matter of the suspended sediment in the fresh water tidal zones. Experiments performed in their laboratories suggest that the metals are released as organometallic complexes.

Muller and Forstner (1974) have questioned the conclusions of DeGroot, DeGoeij and Zegers and claim that most of the reduction in heavy metal concentration in the sediments of the Rhine Estuary is due to dilution of the sediments derived from the Rhine with cleaner North Sea sediments. However they do not totally discount the solubilization mechanism. At this time, however, it is not at all clear which conclusion is correct.

Organic Debris

Very little work has been done on debris from living organisms in streams. Lammers (1967) has used ultracentrifugation to isolate the suspended organic components of streams. In his later work (Lammer, 1975) he isolated the organic debris from the gut of living filter-feeding organisms. Lammers chose several different bivalves for this work. The use of the bivalves allows one to obtain integrated samples of the debris in the stream. The material isolated from the bivalves was then fractionated by ultracentrifugation. In both of the above papers Lammers deals mainly with the viruses, algae, and bacteria that he isolated, however, he does indicate that other organic particles, such as ribosomes were also isolated.

A number of studies have shown that organisms in natural bodies of water concentrate hydrophobic pollutants such as chlorinated hydrocarbon insecticides in their tissue (Sodergran et al, 1972). It would therefore be expected that some of the organic debris in natural waters would contain elevated levels of hydrophobic pollutants, however, there do not appear to be any studies in the literature that deal with this matter.

Organic Colloids

Organic polyelectrolytes that are very similar to the soil humic and fulvic acids have been isolated from surface and ground waters. As Black and Christman (1963) point out, these materials will impart a brownish color to water if present in high enough concentration and indeed many potable water supplies are colored. Day and Felbeck (1974) have shown that the fungus *Aureobasidium pullulans* which is common in sewage, soils and surfaces, exudes a substance similar to fulvic acid. Martin and his coworkers in a series of papers (see Haider et al, 1972 and Martin et al, 1972 for reviews of this work) have shown that soil microorganisms can form humic substances in two ways: by extracellular transformation of plant and animal constituents into humic compounds, and by synthesis of humic precursors from aliphatic compounds. The most common precursors that Martin and his coworkers have detected are phenols which are polymerized by autoxidation or enzymatic reactions, or more likely by both pathways, into humic materials. Similar transformations in sewage and in surface waters would be expected. Thus we may expect that the degradation of many organic wastes will result in the formation of humic materials. During the polymerization process it is possible that resistant pollutant molecules that have not been degraded by the microorganisms will be incorporated into the humic and fulvic acid polymers.

The first part of this discussion has been principally concerned with the relatively insoluble humic acid associated with soils. However, the humic acid salts of the more common monovalent cations are soluble in water. Wershaw and Pinckney, (1973b) have demonstrated that these humic acid molecules form molecular aggregates in solution, the degree of aggregation depending on both pH and concentration. This aggregation phenomenon was detected both in humic acid fractions and in unfractionated salts. Fractionation apparently increased the chemical homogeneity of the fractions by comparison with the unfractionated salts. The aggregates that have been detected in the fractions therefore probably comprise molecules that are chemically more or less similar. However, in an unfractionated sample the aggregates will contain a diversity of different types of molecules. Even molecules that are quite different from humic acids can be incorporated into the aggregates; Wershaw, et al, (1969) have shown that the sodium salt of humic acid can solubilize DDT by incorporating DDT molecules into the sodium humate aggregates (micelles).

Undoubted, this mechanism is responsible for the transport of other relatively insoluble pollutants besides DDT in natural water systems. Ballard (1971) reported an apparent example of this mechanism at work in a natural system. He found that the urea salt or the ammonium salt of humic acid solubilised DDT and carried it down through a soil profile.

In addition to solubilisation reactions soluble humic acid salts and fulvic acids interact with metals to form colloidal organometallic complexes. Much of the early work on colloidal humic material-metal complexes was on iron complexes. Several workers have found that the oxidation of ferrous iron is greatly retarded in many natural waters that contain humic substances (Theis and Singer, 1974).

Whatever the reaction mechanism, it has been well established that humic material stabilizes both ferrous and ferric iron in natural water systems. This has important implications for water treatment, since the standard method of removing excess iron from water is to oxidize it with oxygen and allow it to precipitate as $Fe(OH)_3$. The presence of humic material inhibits these reactions.

A number of more general studies (Rashid and Leonard, 1973; Orlov and Yeroshicheva, 1967; Ong et al, 1970) have shown that humic materials form complexes with copper, cobalt, manganese, nickel, zinc, iron, and aluminum, and solubilise these metals in natural water systems.

Cheam has reviewed the literature on the complexation of copper (II) by fulvic acid. The available evidence indicates that copper is chelated at a bidentate site consisting of a phenolic hydroxl group and an ionised carboxyl group.

Potable water supplies containing humic materials are often decolonized by chlorination. Rook (1974) has shown that chlorination of humic acid solutions in water result in the formation of haloforms, some of which are known or suspected to be carcinogenic (Nicholson, 1977). A survey for haloforms in the water of 80 cities, conducted by the EPA, showed that occurrence of trihalomethanes is widespread in finished drinking waters and is a direct result of chlorination (Stevens et al., 1975; Symons, 1976). The risks that ingestion of these compounds may pose to human health are discussed in Chapter VI.

Summary

Natural organic polyelectrolytes (humic materials) play a major role in the binding and transport of pollutants in natural water systems. These reactions include: (1) adsorption of pesticides, enzymes, and other organic compounds; (2) free radical reactions including charge-transfer reactions between free radicals; (3) ion exchange and complexation reactions between metal ions and ligands or trace organic polyelectrolytes; (9) solubilization reactions in which relatively insoluble compounds are rendered more soluble.

These reactions will lead to the binding of toxic metals and organic compounds to suspended colloidal particles in raw water supplies.

MICROORGANISMS AND SUSPENDED PARTICLES IN WATER

The tendency of microorganisms to form aggregates and to become concentrated at the surfaces of solid particles, rather than to be uniformly and individually dispersed, may have important consequences for thier survival and for their reactions to the various processes of water treatment. It is doubtful that many of these microbial agglomerates will pass through an efficiently operating water treatment process, but a large setment of the population ingests surface water that has had only partial treatment (i.e. disinfection). Under such circumstances these microbial agglomerates constitute potential health hazards.

Unfortunately, there is a lack of scientific information on either the survival of the bacterial component of these microbial-agglomerates after disinfection of surface waters or on their ability to disaggregate and produce infection in the human host after ingestion. Viral aggregates, on the other hand, have a higher resistance to disinfection than free virions and produce infection in susceptible hosts. (See Chapter III, Microbiology, section on Viruses).

Although there is a large body of scientific information from laboratory and field studies on raw water sources with regard to bacterial and viral agglomerates, the same level of scientific attention has not been given to studies of the viability of these agglomerates in finished drinking water or, more importantly, on infection of the human host after ingestion of these agglomerates.

Particulate-Bacterial Interactions

A knowledge of aquatic and terrestrial habitats of microorganisms is essential to the understanding and resolution of problems that arise when man must use the same environment. The potable water environment has been developed by man out of natural aquatic microbial habitats that are continuously fed, in a microbial population already present. This pollution component of the microbial population supplies the more hazardous aspects to the potable water environment because these are the microbial members that have passed through man and other organisms and have, in some instances, been responsible for debilitation or death.

Studies of microbial aggregates in terrestrial environments that ultimately seed the raw water sources have been carried out by Brock (1966, 1974), Cameron (1965-1969), Rice *et al.* (1975), Henrici (1948), Schmidt (1968), Mallette (1963) and Kononova (1966). These studies demonstrated that the most extensive microbial growth takes place in nature on the surfaces of particles and inside loose flocs of solid particles. This occurs because the nutrients required for microbial growth are also adsorbed at the surfaces of these particles. Only a few microorganisms are found *free* in the soil solution or in raw water because of the lack of dissolved nutrients. Thus, chemical and microbiological analyses of water *per se* will not reveal the higher concentrations of organic nutrients and microorganisms present on the surfaces and inside sand, silt, or clay particles that have settled to lower water strata or to the bottom of the raw water sources. Some of the aggregates will be sequestered by sedimentation, but microbial particulates in lake and reservoir sediments remain viable, and grow rapidly at the time of the spring inversion, when mixing with upper layers will cause some redistribution (Henrici, 1948;Boylen and Brock, 1973; Hendricks, 1973).

Mutual microbial interactions also occur not only in association with inorganic and organic particulates but also in their absence to form mixed microbial aggregates. Such mutualistic relationships may benefit both organisms in such ecological associations or show a graded series of symbioses of variable stability and specificity. Studies of these particulate interrelationships have been reviewed by Lederberg (1952) and investigated by Sharp and Church (1963), and Rice *et al.* (1975).

These mutualistic relationships show that microbial growth takes place in environments unsuitable for rapid growth of either member alone; this concept has implications for the survival and rapid growth of microorganisms in nutrient-starved aquatic systems e.g., potable water supplies.

Investigations of the physical-chemical attachment of microorganisms to sand, silt, and clays, the difficulties encountered in disaggregating these particulate complexes, and the conditions promoting migration of the aggregates into potable water supplies (deep wells in particular) have been reported by Stotzky (1965, 1966), Lammers (1967), Boyd *et al.* (1969), and Gray and Wilkinson (1965). Of the physical-chemical characteristics studied, Stotzky in a series of publications, showed that cation exchange capacity (CEC) had an important effect on the binding of organisms to particles. Lammers isolated and fractionated various organic and inorganic particle species occurring in natural waters. Studies of this kind could help to elucidate the composition and concentration of microbial particulates in water.

Particulate-Viral Interactions in Water

River silt adsorbs viruses with moderate efficiency but does not relinquish them very easily. Berg (1973) showed experimentally that silt adsorbed up to 94% of polio virus 1 from distilled water, but only 0.3 to 0.6 percent of the adsorbed virus could be eluted from the silt. He also showed that viruses were recovered more frequently from the silt filtered *fzm* river water than from the river water itself. Since viruses were not recovered so efficiently from silt as from water, there would appear to be much more virus adsorbed to the silt than suspended in the water. These studies on viral adsorption to sand, silt, clays, and organics (feces) to form particulates are consistent with what is known for bacterial aggregates.

To infect by ingestion of water, viruses must pass from their source (human and animal excreta) through two formidable barriers: sewage treatment, and water treatment. In both processes, formation of large particulate - viral flocs are required for settling and filtration. Sewage or water treatment may inactivate the virus, but viruses may survive in the settled sludge or on filters.

Particulate Microbial and Viral Aggregation Increases Survival to Environmental Factors and to Disinfection

A number of studies have demonstrated that the presence of particuate matter in water interferes with disinfection. Neefe *et al.* (1947), Sanderson and Kelly (1962), Symons and Hoff (1975), Tracy *et al.* (1966), Walton (1961), and Hudson (1962) have all showed this phenomenon. This microbial-particulate association must be recognized as a resistant, potentially hazardous, protected, and not easily dissociated complex which remains viable for long periods, and may be present in a water system without showing any easily measured increase in turbidity.

Symons and Hoff (1975) demonstrated the resistance of viruses to disinfection when they were attached to "particulates" compared to "free" virus. Tese studies were carried out for comparative purposes in the presence of 1.0 mg/l of free chlorine residual. Inactivation of virus preparations consisting of artificial mixtures of washed uninfected host cells and purified virus that had been stored at 5°C overnight; virus preparations in which washed host cells were added immediately before addition of purified virus; chlorine demand-free virus; and host cell-associated virus-protective effect was demonstrated in both the natural and artificial cell debris-virus preparations. The viral survival ratio was reduced about three orders of magnitude in *five* minutes. In contrast, results with "free" virus and with virus plus host cells added separately when both were pre-disinfected, showed reductions of greater than five orders of magnitude in *one* minute.

The nature of the association of virus particles with cell debris and the degree to which these preparations approximate the conditions in which viruses are found in nature and in treated water are unknown and research in this area is minimal. Early indications are, however, that certain kinds of particles can protect virus from inactivation by chlorine. Melnick (1975) discusses the viral inactivation problems associated with the use of heat, formalin, chlorine, and other agents, in cases where tissue culture techniques demonstrate viral inactivation (no PFU). If however, the virus is inoculated into an animal host, it may produce infection although no PFU's were demonstrated in tissue culture. (See also Chapter III, Microbiology, section on viruses). Viral particulates and aggregates form protective barriers to some of the internal virions of the aggregate. Thus the nucleic acid component of the virus is doubly protected against disinfecting (inactivating) agents by the viral protein coat and by the outer mass of virus particles in the aggregate.

Petrilli *et al.* (1974) used an experimental treatment plant to show that enteroviruses survived even though coliforms, *E. coli*, and fecal streptococci were eliminated after treatment of raw water by coagulation with ferric chloride, sedimentation, filtration through activated carbon or sand, and terminal chlorination. He also demonstrated survival of enterovirus in spring water that had been chlorinated sufficiently to destroy the coliforms and fecal streptococci tht were also initially present. A review of viral problems facing water treatment plant operators is presented by Taylor (1974).

Conclusion

Investigations are required of the physical-chemical attachment of microorganisms to sand, silt, and clays, and disaggregation of these particulate complexes. Viral aggregates are more resistant to disinfection than are separate viral particles. Fundamental information is needed on the interactions between the viable and nonviable components of particulates in drinking water and particularly on their resistance to disinfecion and to other water treatment processes.

PARTICULATE REMOVAL AND TURBIDITY

Definitions and Occurrence

Some terms commonly used in water treatment practice are described here. *Turbidity* in water is caused by the presence of suspended matter such as clay, silt, non-living organic particulates, plankton, and other microscopic organisms. Operationally, turbidity measurements are expressions of certain light scattering and absorbing properties of a water sample. *Color* in water is due primarily to the presence of natural organic matter; it may also be caused by certain industrial wastes and by some metallic complexes. Color is measured by determining light adsorption. Hence, colloidal particles can produce some color as it is operationally determined.

Filtration has been defined as the passage of a fluid through a porous medium to remove matter held in suspension. Particles ranging in size from a small fraction of a micrometer to several hundred micrometers can be removed by conventional packed-bed filters used in water treatment if appropriate chemical pretreatment is provided. *Coagulation* is a process in which colloidal particles are destabilized by the addition of suitable chemicals and then assembled into larger aggregates primarily by gentle fluid motion. *Sedimentation* is a process by which solids are separated from water by gravity. In conventional treatment for the removal of particulates, the solids in water are first coagulated into compact, fast-settling flocs. Most of these are then removed by sedimentation, after which the water is filtered to remove additional particulates.

Softening involves the removal of calcium and magnesium ions from water. Usually these are removed as solids, viz., calcium carbonate and magnesium hydroxide. Hence, water softening plants have sedimentation and filtration facilities for the removal of these precipitates. Other solids in the raw water (e.g., clay) and other precipitates that may be formed in the softening process can then be removed by these solid-liquid separation processes.

A survey by the USPHS of municipal water facilities in communities with populations of 25,000 or more was summarized by Jenkins (1963). The population covered by this survey totaled 100,940,020 people. A minimum of 62 per cent of this population received filtered water and hence was delivered water treated for the removal of particulates. This information has been revised and expanded to include more communities in a facilities survey by the EPA in the early 1970s. The results are presently being analyzed and summarized by EPA.

Removal of Particulates

Mineral Fibers. Pilot-scale filtration experiments for the removal of amphibole and chrysotile fibers from the Duluth water supply have been conducted (Black and Veatch, Consulting Engineers, 1975; Logsdon and Symons, 1975). Amphibole fibers were easily removed by coagulation and filtration. Considerable difficulties were encountered in removing the chrysotile fibers. These were small and may have been positively charged. Since conventional filters are able to remove submicroscopic particles (Yao et al., 1971), and since conventional pretreatment chemistry (coagulation) is designed to destabilize negative particles, it is plausible that the surface characteristics of the chrysotile particles may hinder their removal by conventional filtration practice. Finally, it is important to note that the design, operation, and evaluation of water treatment facilities for the removal of fibrous particles is seriously impaired by the time, effort, and expense required to detect these particles in water supplies.

Clay. Direct evidence of the removal of clays from water supplies by coagulation and filtration is lacking, since clay particles are not detected directly in routine water analysis. It can be implied that clays are effectively removed since they comprise a significant portion of natural "turbidity", and conventional treatment plants are very effective in turbidity removal. This conclusion is substantiated by laboratory studies of the coagulation and filtration of clay suspensions. Studies of this type are numerous. For example, Packham (1965) and Black and Hannah (1961) have demonstrated effective coagulation and sedimentation of kaolinite, montmorillonite, and other clay suspensions using aluminum sulfate for destabilization under conditions similar to those encountered in potable water treatment. Among many others, Ling (1955) and Adin and Rebhun (1974) demonstrated effective removal of clays from suspension by filtration after appropriate chemical pretreatment.

Organics. Conventional processes for the removal of particulates are effective in removing color and many other organic macromolecules and particulates from water. Here again laboratory studies provide the bulk of the available evidence. Hall and Packham (1965) have demonstrated that humic and fulvic acids can be coagulated by iron (III) and aluminum(III) salts. Humic acids were readily removed, but a significant fraction of the fulvic acid component was not removed by either coagulant. Similar results have been reported by others, including Black and Willems (1961) and Beck (1975).

Microbiological Particulates. Large microorganisms including algae and amoebic cysts are readily removed by filtration from properly pretreated water. Bacterial removals in excess of 99 per cent are also achievable (ASCE *et al.*, 1969). More than 98 per cent of poliovirus Type 1 was removed by conventional coagulation and filtration (Robeck *et al.*, 1962). Complete (i.e., 100 per cent) removal of microorganisms is not feasible, so that filtration is followed by disinfection with chlorine in conventional water treatment practice.

Possible Concerns

Halomethanes and other chlorinated organics. The natural humic substances are known to be precursors for the formation of chloroform and other halomethanes in water treatment. These may be colloidal, or adsorbed on other colloids such as clays. A recent study (Stevens et al., 1975), has indicated that the rate and extent of chloroform production is reduced when chlorine is added to water containing humic substances after filtration, rather than before coagulation. Presumably, some organic materials (particulates, or solutes adsorbed on particulates) are removed by coagulation and filtration prior to chlorination. Hence, water treatment for particulate removal can lead indirectly to reduced chloroform production.

Encasing of Microbial Particulates. It has been proposed that pathogenic bacteria and viruses can be encased in gelatinous metal hydroxides during conventional coagulation processes. If these particles then pass through subsequent settling and filtration facilities, it has been further proposed that the hydroxide gel coatings can protect the pathogens from being inactivated by chlorination. It has also been proposed that natural organic substances can coat and protect pathogens from disinfection. These problems are addressed by Symons and Hoff (1975). Their preliminary evidence suggests that adsorption of virus particles on clays or coagulation of viruses by aluminum sulfate do not impair disinfection of poliovirus Type 1 by chlorine. The presence of cell debris, however, significantly slowed the rate of disinfection. This problem requires additional research.

Coagulant Precipitates. The amorphous hydroxide flocs (e.g., aluminum hydroxide, ferric hydroxide) formed in many coagulation processes are reactive solids. They can, for example, adsorb trace metals and organic compounds. Hence, if they pass through filters and into the treated water, they may carry other more serious substances with them. This is plausible, but conjectural.

Powdered carbon. Powdered activated carbon is frequently used for controlling taste and odor. If some of these carbon particles pass through the filters, they exert a chlorine demand and may also carry adsorbed substances with them. Some of these adsorbed substances might have been on the carbon before it was added to the water. EPA does not at this time have standards for carbon used during water treatment. The problem is at present hypothetical, i.e., no data are available.

Organic Coagulants. Many synthetic organic materials have been approved for use in water treatment for removal of particulates. These have been examined and certified as non-toxic. The problem of the safety of polyelectrolytes is currently under review by EPA and FDA (Symons, 1976).

Conclusions

1. Many harmful substances in raw water supplies and in treated potable waters are either particulates themselves or adsorbed on solid particles.
2. These particles can be removed efficiently by conventional water treatment using coagulation, settling, and filtration processes.
3. Particles that pass through conventional coagulation and filtration plants in significant amounts do so because of inadequate design, operation, or control of these facilities. Some of these particles may be harmful in themselves. Most are innocuous, but have the potential of containing harmful substances.

Turbidity

This consideration of turbidity as an indicator of potable water quality begins with an evaluation of the test itself. Two recent reviews are excellent (McCluney, 1975; Pickering, 1976). Turbidity determinations involve measurements of the light scattering and absorbing properties of a suspension. A variety of definitions, methods of measurement, instruments, standards, and units of measure have been used. Typically, the light scattered at an angle of 90° to an incandescent source beam is determined. The amount of light scattered depends on the number, size, shape, and refractive index of the particles, the wavelength spectrum of the incident light, and the geometry and detection characteristics of the turbidimeter.

A variety of standards (American Public Health Association, 1976) and instruments are in current use. However, each type of instrument will respond differently to the diversity of sizes, shapes, and types of particles found suspended in both raw and treated waters. Furthermore, depending on the method of standardization used, the same instrument can indicate different turbidities on a single water sample. Hence, it is important that the turbidity test be standardized to provide a sound base for the possible use of turbidity as an indicator of water quality and for other uses.

Since many pollutants in water are either particles themselves or are adsorbed on particles, and since particles absorb and scatter light, it is advantageous to consider turbidity as an indicator of water quality. In addition, there are indications that normally innocuous particles can, at least under some circumstances, protect pathogens from a disinfecting agent (Symons and Hoff, 1975). Finally, it is important to note that turbidity determinations are rapid, relatively inexpensive, and can be performed continuously by detectors *in situ*.

Measurements of turbidity do not give complete information about the size, number, mass, or type of the particles that scatter or absorb light. Small particles (those less than about 0.1 um in maximum dimension, single viruses and many asbestos mineral particles) do not scatter much visible light and so are not detected by conventional measurements. Furthermore, larger particles can scatter light effectively but may be harmless; the presence of ice crystals in Arctic waters illustrates this point.

Conclusions

The absence of a detectable turbidity does not guarantee that a water is free from harmful particulates. A determination that a water has a high turbidity does indicate the presence of particulate materials and is a cause for concern.

Turbidity is a collective parameter. It cannot replace specific tests for individual pollutants (e.g., Pb, asbestos); it can provide an indication that such tests should be performed. The nature of the particulates in a raw water supply should be determined, and removal accomplished when necessary, by treatment of the supply and appropriate source control.

The rapidity, low cost, and "*in situ*" features of turbidity measurement make it valuable for monitoring and operation of water treatment plants. When high turbidities are observed in potable water supplies, particular attention should be given to the chlorine demand of the water and to the disinfection process.

Finally, the test itself must be standardised. This will involve the selection of a single light-scattering standard material or a single optical unit, and a standardised light source, instrument geometry, and detection system.

SUMMARY AND CONCLUSIONS

Materials suspended in drinking water include inorganic and organic solids as finely divided particles of sizes ranging from colloidal dimensions to over 100 micrometers. Such particles may also have other substances and microorganisms attached to them.

Small particles of some materials, such as the asbestos minerals, may have the potential to affect human health directly when they are ingested, and their is widespread concern over the biological effects of such substances.

Many kinds of particles, though apparently harmless in themselves, may indirectly affect the quality of water by acting as vehicles for concentration, transport and release of other pollutants.

Water treatment can often be effective in removing most of the particles suspended in it, but conventional methods of detecting the presence of particulate material by measurement of turbidity have serious deficiencies.

Direct Effects on Health

Particles of asbestos and other fibrous minerals occur in raw water; usually in a range of sizes from fractions of a micrometer to a few micrometers. Generally there are fewer than 10 million fibers per liter, but waters are found with from less than 10,000 to more than 100 million fibers per liter. Some of the highest counts have been found near some cities. Fibers in drinking water are typically less than 1 micrometer long and fibers longer than 2 micrometers are uncommon. Identification and counting of fibers is difficult, and time consuming usually requiring the transmission electron microscope. The reported counts are highly variable, often differing from one count to the next by a factor of 10 or more

Epidemiological studies of workers exposed to asbestos by inhalation have shown an increase in death rates from gastrointestinal cancer. With respiratory exposure it is likely that more fibers are swallowed than remain in the lungs. The workers studied were exposed to asbestos with a large range of fiber lengths. It is not clear whether fiber length is pertinent to the development of cancer in the digestive tract in humans.

Epidemiological studies of cancer death rates in Duluth, Minnesota, where the water supply has been contaminated with mineral fiber, have so far not revealed any increase with time (or in comparison with death rates in other areas). Contamination of the Duluth drinking water began less than twenty years ago.

Since many cancers have long latency periods, these negative epidemiological findings do not exclude the possibility that an increase might appear within the next five to fifteen years.

Animal deposition model studies have shown that fiber length and diameter affect the carcinogenic response seen, the long thin fibers appearing to be the active ones. However, the relevance of these animal deposition models to the human experience is not clear. While some animal studies have shown penetration of the gastrointestinal epithelium, others have not.

It is not known whether other inorganic particulates that occur in water produce any direct effects on human health.

Indirect Effects on Health

The concentration of inorganic, organic and biological pollutants is usually much higher in the suspended solids and sediments of streams and lakes than in water. Clay, organic, and biological particulates alone or in combination are the materials chiefly responsible for such concentrations. Clay and organic particulates have large surface areas and strongly adsorb ions, polar and nonpolar molecules, and biological agents. Occurrence of these materials in water is a consequence of natural events, as well as human activity, and is common in many waters that people drink. It is not likely tht many of the clay or natural organic particulates, in themselves, have deleterious effects when ingested by humans. However, they may exert important health effects through adsorption, transport, and release of inorganic and organic toxicants, bacteria and viruses. The clay or organic complex with a pollutant may be mobilized by erosion from the land, or complexes may form when eroded particulate matter enters a stream containing pollutants. The atmosphere is also an important pathway. In the adsorbed state, organic and inorganic toxicants may be less active; however, the possibility exists that the toxicants may be released from the particulate matter in the alimentary tract and then exert toxic effects. It is not clear how complexes of particulate matter with viruses and bacteria behave in the gut. It is known, however, that some enzymes retain their activity when adsorbed on clay, and that viral-clay particulates are infectious in tissue culture and in animal hosts.

Particulate materials, whether inorganic or organic, are usually not in themselves potential health hazards, but may become vehicles for pollutant concentration, transport and release. Thus, entry of pollutants into water that may be used by man should be reduced or prevented, so as to minimize the importance of adsorpion, transport, and release by particulate matter.

Water Treatment for Particulate Removal

Most of the particulates in water can be removed by coagulation, sedimentation and filtration. When appreciable numbers of particles pass through conventional coagulation and filtration plants it is usually because of inadequate design, operation, or control of these facilities. Appropriate coagulation and filtration can provide protection in most cases in which particles pose a possible risk to health.

Turbidity as an Indicator

A high turbidity measurement is an indication that a water may produce an adverse health effect; however, a low turbidity measurement does not guarantee that a water is safe for potable use. Turbidity measurements do not indicate the type, number or mass of particles in a water supply. Where particulates in water are suspected of beig harmful, the particulate content should be identified and counted by more specific techniques. Such techniques may include biological, organic, inorganic, and fibrous particulate surveys.

Turbidity measurements are valuable for process control in water treatment plants. However, present instruments, procedures and units of measurement do not permit reproducibility. The test itself must be standardized to facilitate its use for this and othr purposes.

Conclusions and Recommendations

Certain mineral fibers found in water are suspected of being harmful upon ingestion. The available data with respect to orally ingested asbestos, through drinking water, does not suggest an immediate hazard to public health. It does suggest that additional research, both experimental (using animals), and epidemiological, is required to answer the various questions, and to give further assurance of safety. Until new results become available, contamination of drinking water by mineral fibers must be kept to a minimum through the use of effective coagulation and filtration processes.

As for indirect effects of particulate matter, because hey are vehicles for pollutant concentrations, transport, and release, they become a cause for concern. Coaulation and filtration are effective methods of reducing particulate concentrations. Measurements of particulate content by turbidimetry is imprecise and cannot be relied upon as a sole indicator of the safety of an uncharacterized drinking water source.

Recommendations for Future Research

Following is a list of recommended research: 1) A survey of suspended particulate matter in raw and treated drinking water supplies in several "typical" communities is urgently needed as background information. This must be coupled with analysis of accompanying organic and inorganic material and microorganisms in solution, as well as characterization of the particulates with respect to size, shape, composition and adsorbed constituents. By nature such surveys must be interdisciplinary involving analytical chemistry, surface chemistry, particulate chemistry, organic chemistry, microbiology, etc.

2) Ingestion studies should be carried out with fibers of various size distribution in validated animal model systems.

3) Epidemiological studies of time trends in death rates should be conducted in areas that have high concentrations of mineral fibers in drinking water.

4) The electron microscopy procedures for detecting and counting asbestos fibers should be scrutinized with respect to their specificity, precision and accuracy. This and other techniques and combinations should be investigated for use in screening.

5) Information is required on the effects of inorganic, organic and biological toxicants adsorbed on clay and organic particulates. Such work should be interdisciplinary in nature; expertise on surface chemistry of clays and organic particulates as well as in microbiology, toxicology and physiology are required.

6) Development of improved and standardized methods for measuring turbidity should be supported.

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Chapter V

INORGANIC SOLUTES

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Chapter V
INORGANIC SOLUTES

TRACE METALS

Trace metals may be present in natural ground or surface water. The sources of these trace metals are associated with either natural processes or man's activities. Two important natural processes contributing trace metals to natural water are chemical weathering and soil leaching. The factors affecting the release of trace metals from primary materials and soil and their solution and stability in water are solubility, pH, adsorption characteristics, hydration, coprecipitation, colloidal dispersion, and the formation of complexes. Decaying vegetation can also affect the concentration of trace metals in water. Many plants are known to concentrate various elements selectively. As a result, trace metals may become available during the decay of the plants. Thus, the penetration and movement of rainwater through soil may pickup these available trace metals and affect the ground-water resource. Likewise, runoff resulting from rainfall may transport trace metals to surface water.

Mining and manufacturing are other important sources of trace metals in natural waters. Several operations associated with the mining of coal and mineral ores can lead to the discharge of wastewater contaminated with trace metals or to the accumulation of spoiled material, which may be leached of trace metals by rainfall and reach either surface or groundwater. The discharge of industrial wastewater, such as that generated by plating and metal-finishing operations, may also be the source of trace metals in natural water.

The treatment of raw surface or groundwater to make it acceptable for public consumption may include the removal of trace metals. However, trace metals may be added to water as a result of the treatment and the subsequent distribution throughout a community. Depending on the quality of the raw water and the quality desired in the finished (treated) water, treatment may involve the use of chemicals, such as alum (aluminum sulfate), lime, and iron salts. The chemicals used are usually of commercial or technical grade with no exact composition, although the American Water Works Association has established standards for most chemicals used in the treatment of water supplies. Because of the possibility of impurities in the chemicals, it is conceivable that trace metals may be added to the water during treatment. A chemical itself, such as alum, may also contribute to the trace metal content of the finished water, depending on its solubility and the characteristics of the water.

The occurrence of corrosion in the distribution system may also add trace metals to finished water before it reaches the consumer. Common piping materials used in distribution systems are iron, steel, cement (reinforced concrete), asbestos cement, and plastic. Lead, copper, zinc, aluminum, and such alloys as brass, bronze, and stainless steel may also be used in addition to ferrous metals in pumps, small pipes, valves, and other appurtenances. Trace metals may be contributed to the water through corrosion products or simply by solution of small amounts of metals with which the water comes into contact.

Trace Metals in Water Samples Collected in the Distribution System or at Household Taps

The concentration of trace metals in water collected in the distribution system or at household taps, is more relevant with respect to the quality of water being consumed by the public than is the raw water. The data in Table V-1, taken from the community water supply survey involving 969 public water supplies, indicate that the level of several selected elements in water samples collected in distribution systems were present in microgram quantities (McCabe *et al.*, 1970). However, the maximum levels of some metals--cadmium, lead and barium--were found to be in the milligram range.

The results of analyzing a number of tap-water samples, collected at homes in Dallas, Texas, for trace metals are given in Table V-2. In the unpublished report from which these data were taken, it was speculated that the high iron concentration was due to the use of steel water mains in the distribution system, whereas the high manganese concentration was the result of accumulation of sandy sediment in the distribution system. The high copper and zinc concentrations in the water samples were believed due to the household plumbing. "Local influences" was the reason cited for the high lead and nickel concentrations in the tap water.

Several studies have shown the combined effect of treatment and the distribution system on the trace-metal content of the water reaching consumers. A treatment plant handling 90 million gallons/day (90 mgd) and obtaining its raw water from the Allegheny River was studied with respect to barium, copper, and nickel (Shapiro *et al.*, 1960). This particular plant used sedimentation, slow sand filtration, and chlorination. Water samples were collected for analysis before and after chlorination and at a consumer's tap at a remote point in the distribution system. Nickel and copper occurred in significantly higher concentrations in the tap water, compared with the treatment plant after chlorination--100 μg/liter vs. 30 μg/liter for nickel and 4,000 μg/liter vs. 90 μg/liter for copper. In the case of barium, the concentration was lower at the tap--40 μg/liter vs. 90 μg/liter. Following chlorination the concentration of copper in the water was always found to be higher--from 30 μg/liter before to 90 μg/liter after.

TABLE V-1

Concentrations of Selected Trace Metals in 2,595 Distribution Water Samples

Element	Limit* mg/liter	Maximum Concentration, mg/liter	Fraction of Samples Exceeding Limit, %
Barium	1.0	1.55	<0.1
Cadmium	0.01	3.94	0.1
Chromium (VI)	0.05	0.08	0.2
Lead	0.05	0.64	1.4
Silver	0.05	0.03	0

*USPHS Drinking Water Standards of 1962

(From McCabe _et al_., 1970)

TABLE V-2

Concentrations of Selected Trace Metals in Household Tap Water Samples, Dallas, Texas

Element	No. Samples	Concentration, mg/liter			
		Average	Median	Maximum	Minimum
Cadmium	43	0.011	0.003	0.056	0.001
Chromium	36	0.006	0.003	0.020	0.001
Copper	43	0.037	0.029	0.164	0.004
Iron	35	0.097	0.080	0.278	0.031
Mercury	43	0.000115	0.000100	0.000845	0
Manganese	43	0.0037	0.006	0.008	0.001
Nickel	36	0.0109	0.010	0.023	0.005
Lead	43	0.0095	0.010	0.027	0
Zinc	43	0.0124	0.011	0.049	0.005

TABLE V-3

Comparison of Concentrations of Several Trace Elements in Raw and Tap Water of Three Cities in Sweden

Element	Raw-Water Concentration, µg/liter	Concentration Ratio, Tap:Raw		
		Malmo	Stockholm	Goteborg
Barium	1-3	6.7	4.0	0.5
Cadmium	0.02-0.3	2.5	0.5	1.0
Cobalt	0.1	0.7	1.0	3.0
Copper	2-13	0.5	0.2	0.4
Mercury	0.09-0.4	1.1	1.0	1.0
Zinc	8-28	4.5	2.8	1.4

(From Bostrom and Wester, 1967)

The effect of treatment and the distribution system on the concentration of trace metals was also studied in three cities in Sweden--Malmo, Stockholm, and Goteberg (Bostrom, and Wester, 1967). A comparison of the raw and tap water concentrations of six trace metals is shown in Table V-3.

The change in concentration of several trace metals in raw, finished, and tap water was studied in the Denver municipal system, which draws its raw water from a variety of sources and uses five treatment plants that are interconnected, which makes it impossible to determine the plant from which a tap-water sample is derived (Barnett *et al.*, 1969). The maximum:minimum ratio for most of the trace metals in the raw water varied from 1.5:1-6.5:1; higher ratios were observed for aluminum, iron, molybdenum, and zinc. A comparison of the concentrations of the trace metals in the tap and finished water, based on ratios, shows that there were both reductions and increases in the distribution system. As with the raw waters, the concentrations of trace metals in the tap-water samples showed considerable variation.

A distribution system in Seattle, Washington, was studied in an attempt to determine the severity and location of the corrosion that was known to be occurring (Dangel, 1975). The concentrations of several trace metals were determined in the raw water and in two samples collected at household taps. Standing samples were collected as soon as the tap was turned on; this represented water in contact with the household plumbing at least overnight. Running samples collected after bleeding the line for 30 s represented water from the distribution main. The corrosiveness of the system was recognised by the low pH, acidity, and hardness of the water. A comparison of the concentrations of iron, copper, zinc, lead, and cadmium in the raw water with those in the standing water confirmed the corrosiveness of the water. However, after a comparison of the concentrations of the same trace metals in the standing and running samples, it was concluded that most of the metal pickup was occurring in the service lines connecting the distribution main to the buildings and in the inside plumbing. It was also noted that the corrosion products tested--the trace metals--correlated well with the materials in contact with the water.

Trace Metals in Finished Water Supplies

A survey of the mineral content of the water served to customers (finished water) in the 100 largest U.S. cities was made in 1962 (Durfor and Becker, 1964). The highest, median, and lowest concentrations are listed in Table V-4. The raw water used by these cities was either groundwater (wells and infiltration galleries) or surface water (streams, reservoirs, and lakes). The chemical quality of most groundwater supplies is stable, compared with that of streams, whose quality often varies seasonally. Streams are generally impounded during flood periods; the mineral content of water in reservoirs is generally less than that of water in unimpounded streams.

In addition to the quality of the raw water, it is important to recognize that water-treatment practices can affect the concentration of trace metals in finished water. This can be seen from the data in Tables V-5 and V-6. The concentrations of several trace metals in surface water of the United States are summarized in Table V-5. Table V-6 gives values for finished municipal water after treatment. This summary of analyses performed on raw surface water and finished water indicates higher mean concentrations of iron, zinc, lead, copper, and aluminum in finished water. This broad comparison points to the possibility that trace metals are added to water during treatment. Barnett *et al.* (1969) cited such an instance in which the use of aluminum sulfate at a treatment plant increased the aluminum concentration in the finished water by a factor of 5, compared with the raw water. Shapiro *et al.* (1962) observed, in a study of Pittsburgh tap water, a considerable increase in the copper content between samples at the water-treatment plant and those taken in the distribution system. Nickel also showed a tendency to be higher in the distribution water samples than at the treatment plant; however, the opposite was true for barium.

In comparing the concentrations of several trace metals in raw water taken from the Thames River and finished water at two treatment plants using prechlorination, flocculation with alum, rapid sand filtration and post chlorination, it was found that treatment had no effect on the cobalt concentration (Andelman and Shapiro, 1973). However, as a result of treatment, the concentrations of manganese and nickel in the finished water decreased, whereas those of copper and cadmium increased.

In addition, 83 water-supply systems in EPA Region V were examined for various organic and inorganic constituents (USEPA, 1975). Region V consists of Illinois, Indiana, Michigan, Minnesota, Ohio, and Wisconsin. The water supplies examined were selected jointly by the EPA and the states and consisted of 14 groundwater and 69 surface-water supplies. The concentrations of metals in the raw- and finished-water supplies included in the survey are summarized in Table V-7.

TABLE V-4
Maximum, Minimum, and Median Concentrations of Constituents of Finished Water in Public Water Supplies of 100 Largest Cities in United States

Constituent	High	Concentration,* mg/liter Median	Low
Iron	1.3	0.02	0.00
Manganese	2.5	0.00	0.00
Magnesium	120	6.25	0.00
Silica	72	7.1	0.00
		µg/liter	
Silver	7.0	0.23	ND
Aluminum	1,500	54	3.3
Barium	380	43	1.7
Chromium	35	0.43	ND
Copper	250	8.3	<0.61
Molybdenum	68	1.4	ND
Nickel	34	<2.7	ND
Lead	62	3.7	ND
Vanadium	70	<4.3	ND

ND, not detected.
(From Durfor and Becker, 1964)

TABLE V-5
Frequency of Detection and Concentrations of Dissolved Trace Metals in 1,577 Raw Surface Waters in the United States (October 1, 1962-September 30, 1967)

Element	Frequency of Detection, %	Concentration, µg/liter		
		Minimum	Maximum	Mean
Zinc	76.5	2	1,183	64
Cadmium	2.5	1	120	9.5
Iron	75.6	1	4,600	52
Molybdenum	32.7	2	1,500	68
Manganese	51.4	0.3	3,230	58
Aluminum	31.2	1	2,760	74
Beryllium	5.4	0.01	1.22	0.19
Copper	74.4	1	280	15
Silver	6.6	0.1	38	2.6
Nickel	16.2	1	130	19
Cobalt	2.8	1	48	17
Lead	19.3	2	140	23
Chromium	24.5	1	112	9.7
Vanadium	3.4	2	300	40
Barium	99.4	2	340	43

(From Kopp, 1970)

TABLE V-6
Frequency of Detection and Concentrations of
Trace Elements in 380 Finished Waters in the
United States (October 1, 1962-September 30, 1967)

Element	Frequency of Detection, %	Concentration, µg/liter		
		Minimum	Maximum	Mean
Zinc	77.0	3	2,010	79.2
Cadmium	0.2	12	12	12
Iron	83.6	2	1,920	68.9
Manganese	58.7	0.5	450	25.5
Copper	65.2	1	1,060	43
Silver	6.1	0.3	5	2.2
Lead	18.1	3	139	33.9
Chromium	15.2	1	29	7.5
Barium	99.7	1	172	28.6
Molybdenum	29.9	3	1,024	85.9
Aluminum	47.0	3	1,600	179.1
Beryllium	1.1	0.02	0.17	0.1
Nickel	4.6	1	490	34.2
Cobalt	0.5	22	29	26
Vanadium	3.4	14	222	46.1

(From Kopp, 1970)

TABLE V-7

Metal Concentration Ranges in Raw- and Finished-Water Supplies of 83 Cities in EPA Region V

Element	Concentration, µg/liter Raw Water	 Finished Water
Silver	<0.2-0.3	<0.2-0.3
Arsenic	<1.0-10.0	<1.0-50.0
Cadmium	<0.2-12	<0.2-0.4
Chromium	<5.0-17.0	<5.0-6.0
Copper	<5.0-200.0	<5.0-200.0
Iron	<20-330	<20-1100
Magnesium	1800-63,000	800-49,000
Manganese	<5.0-760	<5.0-350
Sodium	1100-77,000	1000-91,000
Lead	<2.0-30.0	<2.0-20.0
Selenium	<5.0	<5.0
Zinc	<5.0-210	<5.0-460

Occurrence of Trace Metals in Raw Water Supplies

In reporting the results of various water surveys, no attempt has been made to distinguish between different analytical methods used which may well have different sensitivities and precision.

Barium. Barium was found in 99.4% of the surface-water samples examined by Kopp and Kroner (1967). The range was 2-340 μg/liter, and the average was 43 μg/liter.

Beryllium. The maximum beryllium concentration observed in 1961 by Durum and Haffty was less than 0.22 μg/liter in the Atchafalaya River at Krotz Springs, Louisiana. Kopp and Kroner (1967) noted the presence of beryllium in 5.4% of their samples, with concentrations ranging from 0.01-1.22 μg/liter and an average of 0.19 μg/liter.

Cadmium. Groundwater contamination from electroplating operations has been reported by Lieber (1954) to cause cadmium concentrations of up to 3.2 μg/liter. In Illinois surface waters, 10 of 27 sampling stations on different watersheds had cadmium concentrations below 10 μg/liter; the maximum observed by Ackermann (1971) was 20 μg/liter. Of 112 samples of surface and ground water in Canada examined, only four had detectable concentrations of cadmium, i.e., 10 μg/liter (Procter and Gamble, 1974). Kopp and Kroner (1967) reported that 2.5% of the surface-water samples examined in their study contained cadmium at 1-120 μg/liter, with a mean of 9.5 μg/liter. In a comprehensive study of U.S. rivers in 1974 (USGS, 1974), a maximum dissolved concentration of cadmium of 42 μg/liter was reported for the Tanana River in Alaska. Durum *et al.* (1971) reported cadmium concentrations of 1-10 μg/liter in 42% of the surface water samples examined, with only 4% above 10 μg/liter; the maximum concentration was 130 μg/liter. High concentrations were reported to occur in densely populated areas. Durum (1974) reported a distinct regional pattern: areas with many pollution sources and higher rainfall were higher in cadmium.

Chromium. Durum and Haffty (1961) reported a range of concentrations for chromium in U.S. rivers of 0.7 to 84 μg/liter. Kopp and Kroner (1967) detected chromium in 24.5% of the samples examined, with concentrations ranging from 1 to 112 μg/liter and averaging 9.7 μg/liter. In a study of surface and ground water in Canada, all but two of 240 samples examined were below 50 μg/liter (Procter & Gamble, 1974). In 1974, a maximum dissolved chromium concentration of 30 μg/liter was recorded in water from the Pecos River, New Mexico; the Los Angeles River; and the Columbia River, Oregon (USGS, 1974). In a 1970 survey, 11 of 700 samples had chromium concentrations of 6 to 50 μg/liter, with none exceeding 50 μg/liter (Durum *et al.*, 1971). Ackermann

(1971) reported chromium concentrations below 5 µg/liter for 18 of 27 river stations in Illinois; the maximum was 50 µg/liter.

Cobalt. The limit of solubility of cobalt in normal river water is approximately 5 µg/liter, according to Durum et al. (1971), who reported that 37% of the river-water samples examined contained cobalt at 1-5 µg/liter, with less than 1% exceeding 5 µg/liter. A 1961 study showed a maximum of 5.8 µg/liter in the Mississippi River at Baton Rouge (Durum, 1961). A recent survey detected a maximum of 17 µg/liter in the Kentucky River at Lockport (USGS, 1974). Kopp and Kroner (1967) found cobalt in 2.8% of surface-water samples examined; the concentration ranged from 1 to 48 µg/liter, with a mean of 17 µg/liter.

Copper. Copper has been observed to adsorb to colloidal material at alkaline pH (McKee and Wolf, 1963). Durum and Haffty (1961) found the maximum copper concentration in the Susquehanna River to be 105 µg/liter. Kopp and Kroner (1967) detected copper in 74.4% of the surface-water samples examined; the concentration ranged from 1 to 130 µg/liter, with a mean of 15 µg/liter. A recent survey detected a maximum of 40 µg/liter in the North Platte River (USGS, 1974). Analysis of 13 Canadian surface and groundwaters--including wells, rivers, and lakes--showed copper at 20-860 µg/liter, the maximum being recorded in Lake Ontario (Proctor & Gamble, 1974). Copper in excess of 100 µg/liter was reported in eight of 27 Illinois streams, with a maximum of 260 µg/liter (Ackermann, 1971).

Lead. Pickering and Henderson (1966) reported a maximum solubility of lead of 500 µg/liter in soft water and 3 µg/liter in hard water. Durum and Haffty (1961) reported a maximum lead concentration of 55 µg/liter in the St. Lawrence River at Levis, Quebec. In a more recent sampling of 727 U.S. sites, lead was found, at 1-50 µg/liter, in 63% of the surface-water samples examined (Durum et al., 1971). However, lead was detected less frequently at U.S. Geological Survey benchmark stations than at locations in more developed areas.

In 1974, the Mississippi River at Vicksburg showed a maximum lead concentration of 29 µg/liter (USGS, 1974). Of 52 surface and groundwaters examined in Canada, 50 were found to have less than 10 µg/liter; the concentrations in the other two samples were 22 and 25 µg/liter (Procter & Gamble, 1974). In Illinois surface water, 25 of 27 river stations were found to have lead below 50 µg/liter; the other two had concentrations greater than 50 µg/liter (Ackermann, 1971). Kopp and Kroner (1967) found lead--at 2-140 µg/liter, with a mean of 23 µg/liter--in 19.3% of their surface-water samples. Durum (1974) reported that the concentration of lead in water, like that of cadmium, can be correlated with urbanization and runoff.

Manganese. Durum and Haffty (1961) observed a maximum manganese concentration of 181-185 μg/liter in two different surface waters. The median for all samples was 20 μg/liter. Kopp and Kroner (1967) detected manganese in 51.4% of surface-water samples; the concentration ranged from 0.3 to 3230 μg/liter, with a mean of 59 μg/liter. A maximum of 1,200 μg/liter was detected in two different surface waters in 1974 (USGS., 1974).

Mercury. Durum *et al.* (1971) found dissolved mercury ranging from 0.1-4.3 μg/liter in 7% of the surface-water samples examined; in some cases, total mercury exceeded 5 μg/liter. According to a survey performed by Jenne (1972), only 4% of the surface waters examined showed mercury in excess of 10 μg/liter; most of these were small lakes and reservoirs. The same study reported that groundwater samples were below the limit of detection for mercury. In 1974, the Rio De La Plata River, Puerto Rico, was observed to have a maximum dissolved mercury concentration of 3 μg/liter, and the James River in Virginia showed 1.6 μg/liter (USGS, 1974).

Molybdenum. Durum and Haffty (1961) detected a maximum molybdenum concentration of 6.9 μg/liter in the Colorado River, Yuma, Arizona. In a more extensive survey, Kopp and Kroner (1967) found molybdenum in 32.7% of their surface-water samples; the concentration ranged from 2-1,500 μg/liter, with a mean of 68 μg/liter.

Nickel. A maximum nickel concentration of 71 μg/liter was observed in the Hudson River at Green Island, New York (Durum and Haffty, 1961). Kopp and Kroner (1967) found nickel in 16.2% of surface-water samples; the concentration ranged from 1-130 μg/liter, with a mean of 19 μg/liter. In a study of 13 Canadian surface- and groundwater resources, only one sample was found to have nickel above the detection limit of 100 μg/liter (Procter & Gamble, 1970). In a study of Illinois surface waters, 36 river stations had nickel concentrations below 50 μg/liter, and three had concentrations of 50-510 μg/liter (Ackermann, 1971).

Silver. Samples containing silver at approximately 1 μg/liter were noted by Durum and Haffty (1961) in the St. Lawrence River, Levis, Quebec, and in the Colorado River, Yuma, Arizona. Of the surface-water samples examined by Kopp and Kroner (1967), only 6.6% contained detectable amounts of silver; the concentration ranged from 0.1-38 μg/liter, with an average of 2.6 μg/liter.

Vanadium. A high vanadium concentration of 6.7 μg/liter has been reported in the Sacramento River, Sacramento, California (Durum and Haffty, 1961). Kopp and Kroner (1967) observed detectable concentrations in 3.4% of the samples analyzed; the concentration ranged from 2-300 µg/liter, with an average of 40 µg/liter.

Zinc. The early studies of Durum and Haffty (1961) showed a maximum zinc concentration of approximately 144 µg/liter in the St. Lawrence River, Levis, Quebec. Kopp and Kroner (1967) found zinc in 76.5% of their surface-water samples; the concentration ranged from 2-1,183 µg/liter, with an average of 64 µg/liter. Durum *et al.* (1971) reported that zinc concentrations as high as 50 µg/liter could be found in surface water in mining areas, but that most samples had a concentration ranging from 10-50 µg/liter. Lazarus *et al.* (1970) reported the average concentration of zinc in rainfall of about 107 µg/liter.

In 1974, a zinc concentration of 730 µg/liter was found in the North Platte River, Lisco, Nebraska (USGS, 1974). In a variety of surface- and ground-water sources in Canada, the zinc concentration was found to be 20-110 µg/liter (Procter & Gamble, 1974). In a study of 27 Illinois surface-water sources, a maximum concentration of 2000 µg/liter was observed (Ackermann, 1971). Durum (1974) reported that the concentration of zinc in surface water, like those of lead and cadmium, could be correlated with urbanization and runoff.

Geographical and Local Factors. Durum and Haffty (1961) studied 15 stations on various rivers. Considering 13 of the trace metals pertinent to this review, 3 of the 15 stations had the maximum concentrations of more than one element. The St. Lawrence River at Levis, Quebec, had the maximum concentrations of silver, lead, and zinc; the Colorado River at Yuma, Arizona, had the maximum concentrations of silver and molybdenum; and the Mississippi River at Baton Rouge, Louisiana, had the maximum concentrations of cobalt, iron, and manganese.

Kopp and Kroner (1967) presented data for 5 years for 16 major river basins in the United States. Table V-8 summarizes the basins in which the highest and lowest 5-year means were reported. The variability between the high and low means is shown as a ratio; for example, the ratio for manganese shows more variability than that for any of the other trace metals for which there was a detectable minimal concentration.

Table V-9 shows the highest and lowest observed concentrations of various trace metals in different surface and groundwaters, as reported in the references cited here. The possible frequency of detection is also given. For example, zinc will, in all probability, be found in 75% of all water samples examined for zinc from various locations, and its concentration will range from 2-50,000 µg/liter.

TABLE V-5
Minimum and Maximum 5-Year Surface Water Concentrations
and Their Geographical Occurrence

Metal	Low Mean, µg/liter	High Mean, µg/liter	High:Low Ratio	River Basins Low Mean	 High Mean
Zinc	16	205	12.8	California	Lake Erie
Cadmium	0	50	c	Tennessee Missouri Low Mississippi California Alaska	Lake Erie
Iron	19	173	9.1	North Atlantic	Western Gulf
Molybdenum	19	185	9.7	South East	Great Basin
Manganese	2.3	232	101	W. Great Lakes	Ohio River
Aluminum	11	333	30.3	Alaska	Western Gulf

(continued)

TABLE V-8 (continued)

Metal	Low Mean, µg/liter	High Mean, µg/liter	High:Low Ratio	River Basins Low Mean	 High Mean
Beryllium	0	0.28	∞	Upper Miss., S.W. Low Miss., Colorado Western Gulf California Great Basin Alaska	Ohio River
Copper	7	23	3.3	W. Great Lakes	Ohio River
Silver	0.3	5.8	19.3	Great Basin	Colorado
Nickel	3	56	18.7	Western Gulf	Lake Erie
Cobalt	0	36	∞	Tennessee Western Gulf California Great Basin Alaska	S.W. Low Miss.
Lead	4	39	9.8	Western Gulf California	Lake Erie Missouri
Chromium	4	25	6.3	South East Great Basin	Western Gulf
Vanadium	0	171	∞	Tennessee W. Great Lakes Great Basin	Missouri
Barium	15	90	6.0	W. Great Lakes	S.W. Low Miss.

(From Kopp and Kroner, 1967)

TABLE V-9
Overall Minimum and Maximum Metal Concentrations in Ground and Surface Waters and Probable Frequency of Detection

	Highest Observed Concentration, µg/liter	Lowest Observed Concentration, µg/liter	Probable Frequency of Detection,* %
Barium	340	2	99+
Beryllium	1.22	0.01	80
Cadmium	120	1	5-80
Chromium	112	0.7	25-65
Cobalt	48	1	5-40
Copper	280	1	75
Lead	140	1	25-65
Manganese	3,230	0.3	50
Mercury	10	0.1	10
Molybdenum	1,500	2	30
Nickel	130	1	25-65
Silver	38	0.1	10
Vanadium	300	2	5
Zinc	50,000	2	75

*Estimated upward from the best values reported in the literature, on basis of current analytic methods.

Removal Of Metals By Water-Treatment Processes

Beyond health considerations, the necessity of removing metals from drinking water is primarily a function of adequate surveillance and the development of analytical procedures capable of detecting trace concentrations. If one is unaware of the presence of trace amounts of a potentially harmful metal in a water supply, the immediate need for its removal does not exist. The need to remove metals raises the question of how effective the current water processes are in removing metals from a water supply. Most treatment processes in use today were not developed to remove trace concentrations of metal. Chemical coagulation-flocculation, for example, is used primarily to remove turbidity and color from raw water; and any significant removal of lead through coagulation with alum is secondary to the original objective. Every treatment plant that uses alum coagulation will vary with respect to its potential for removing lead, owing to differences in water characteristics and operating procedures.

Table V-10 indicates the potential of several different treatment processes for removing barium, cadmium, chromium, cobalt, copper, lead, magnesium, manganese, mercury, methylmercury, molybdenum, nickel, silver, tin, vanadium, and zinc. The treatment processes considered include chemical coagulation (alum and ferric chloride), lime softening (low lime and excess lime), the application of activated carbon, reverse osmosis, and ion exchange. Removal efficiencies have been rated semiquantitatively as "poor" (<30% removal), "fair" (30-60% removal), "good" (60-90% removal), and "very good" (>90% removal). Unfortunately, some studies have been performed whose published reports did not give percentage values for removal efficiencies. In these cases, efficiency was assigned on the basis of the written description.

Chemical Coagulation. Salts of trivalent aluminum and iron have long been used to remove color and turbidity from water. Two mechanisms for the removal of trace metals by aluminum and ferric salts have been proposed: chemisorption to insoluble $Al(OH)_3$ and $Fe(OH)_3$, and by association with organic matter and clays, which are normally removed in the coagulation-flocculation process (Singer, 1974).

Symons et al. (1975) used jar tests with Cincinnati tap water to which trace amounts of various metals were added and reported very good removal of cadmium with ferric sulfate when the pH of the solution was above 7.5. Removal of cadmium with alum was reported as only poor to fair. Removal of barium was expected because of the formation of insoluble barium sulfate. However, only poor results were achieved with ferric sulfate and alum, presumably because of the supersaturation of barium sulfate.

Ferric sulfate and alum concentrations between 20 and 100 mg/liter removed only small amounts of inorganic mercury (II) and methylmercury. Slightly better results were obtained in removing inorganic mercury with ferric sulfate than with alum. Increased removal of inorganic mercury was observed when the suspended solids in the test water were increased. It was speculated that this increase was due to the adsorption of mercury to the particulate matter (Logsdon and Symons, 1973).

Using samples of tap water and wastewater with added doses of various metals, Nilsson (1971) found that lead and copper were removed very effectively by alum doses of 100 mg/liter when the pH was 6.5-7.0. Zinc, nickel, and cobalt were only slightly removed under those conditions. These results were explained by the insolubility of the metals at neutral pH. Lead and copper were suspected to be present as insoluble hydroxides, oxides, and carbonates, which are readily flocculated by alum.

Nordell (1961) noted that coagulation of colloidal oxides of iron and manganese with ferric salts may give favorable results. However, the preferred method of removal consists of aeration followed by settling and filtration if reduced species of iron and manganese are present, which is usually the case. Removing dissolved silica by treating surface waters with ferric sulfate may be effective. Aluminum removal to approximately 1 mg/liter may be achieved by coagulation with alum when the pH of the water is 5.5-6.5.

Poor removal of radioisotopes of chromium, molybdenum, and cobalt by chemical coagulation with Al(III) and Fe(III) salts has been reported by Straub (1964). Coagulant doses of approximately 20-100 mg/liter at neutral pH values were used in jar tests. Increased removal was demonstrated when artificial turbidity was added to the test water, indicating adsorption to the particulate matter.

Lime Softening. The major objective of lime softening is the removal of hardness from water. Two types of softening processes are used: when the alkalinity of water is sufficiently high, the low-lime process is used, and the pH of the water is raised to approximately 9.5-10.5; when the alkalinity of water is low, excess lime may be used to remove hardness not associated with alkalinity, and the pH of the water may be raised to around 10.5-11.5. Removal of trace metals during either process may be due to precipitation as hydroxides at the increased pH or to chemisorption of the metals to calcium carbonate and magnesium hydroxide precipitates.

Symons *et al.* (1975) showed that, although removal of barium by coagulation was poor, good to very good results could be obtained with lime precipition. Cadmium removal with both the low- and excess-lime processes was in excess of 90%. The removal of methylmercury was described as poor and the removal of inorganic mercury was fair to good in studies of both lime processes with the jar test procedure. Additional results presented by the authors indicated that significantly better results were obtained for the removal of both methylmercury and inorganic mercury in a pilot-plant operation.

Lime precipitation of municipal secondary effluent with added trace metals at a pH of 11 was shown to remove silver (50 μg/liter) and cadmium (10 μg/liter) in excess of 95% (Lindstedt et al., 1971). Removal of chromium (50 μg/liter) was very poor. At a pH of 11, silver and cadmium were found to be present as insoluble hydroxides, and chromium as the soluble chromate, CrO_4^{-2}.

Nilsson (1971) demonstrated that--although cobalt, nickel, and zinc were poorly removed by chemical coagulation--lime precipitation at a pH of 9.5 was very effective in removing these metals from samples of tap water and wastewater to which they were added.

In a review of the chemistry of lime precipitation, Dye and Tuepker (1971) emphasized that the removal of magnesium was most effective with the excess-lime process. The removal of magnesium by the low-lime process is usually poor, because of the dissociation of magnesium hydroxide $Mg(OH)_2$ at pH values below 10. Removal of iron by the low- and excess-lime processes and removal of manganese by the excess-lime process were reported to be incidental, because of the high pH associated with each process (Nordell, 1961). Naylor and Dague (1975) found that the excess-lime process with pH greater than 10.5 was unable to remove lead, either because of the physical character of the lead oxides or because of the presence of soluble lead hydroxide $Pb(OH)_4$. Very good removal of lead by the low-lime process was noted. The presence of suspended matter increased the removal of lead with both the low- and excess-lime processes.

Activated Carbon. Activated carbon is normally used to remove substances that cause taste, odor, and color in water. The use of activated carbon for removal of organic matter in general has been recognized. The removal of metals by activated carbon may be due to several mechanisms. Impurities in activated carbon, especially oxygen and sulfur, may play a significant role. Also surface oxides may act as weak-acid cation-exchange sites or sulfide groups may interact strongly with some metals resulting in chemisorption. Activated carbon may also act as a nucleation site for the precipitation of metals. On the other hand, trace metals associated with organic matter may be removed by interactions between the activated carbon and the organic matter. Activated carbon can also act as a reducing agent. Reduction of metal oxyanions--e.g., $Cr_2O_7^{-2}$ and MnO_4^{-2} to Cr(III) and Mn(II)--may result in the precipitation of the reduced species as oxides or hydroxides--$Cr(OH)_3$ and MnO_2 (Singer, 1974).

Logsdon and Symons (1973) and Symons *et al.* (1975) have reported that trace amounts of inorganic mercury and methylmercury can be removed effectively by activated carbon. The superior removal of methylmercury, compared with inorganic mercury, was attributed to interactions between the activated carbon and the methyl functional group. Poor removal of barium with activated carbon was reported.

Lindstedt *et al.* (1971) reported removal of silver, cadmium, and chromium (in excess of 95%) from municipal secondary effluents with activated carbon. The high degree of removal was attributed to a combination of mechanisms.

Sigworth and Smith (1972) extrapolated several years of data on removal of several metals from mill waste solutions by activated carbon to obtain what they felt to be reasonable removal efficiencies that may be expected in the treatment of drinking water. The data were collected for solutions having very high concentrations of metals and low pH values. The authors concluded that the removal of zinc would be poor under the extrapolated conditions, the removal of vanadium and nickel would probably be fair, and the removal of cobalt, iron, lead, and tin would probably be good.

Reverse Osmosis. Reverse osmosis is used as a desalination or demineralization process. The ionic strength of water is reduced by forcing it to diffuse through a cellulose acetate membrane against the high osmotic pressure caused by ionic imbalance. Furukawa (1973) has reported the rejection (separation) of cadmium, chromium, copper, aluminum, iron, magnesium, nickel, and silver by reverse osmosis to be in excess of 98%.

Ion Exchange. Ion exchange involves the reversible exchange of ions between a solution and an exchange resin. Exchange resins are available to exchange either anions or cations. The chemical nature of the resin will also dictate a selectivity for specific ions over other ions, according to the ionic charge, the hydrated ionic radius of the ions, and their concentration in solution.

Linstedt *et al.* (1971) demonstrated the removal of silver and cadmium from municipal secondary effluents by a cation-exchange bed. Chromium VI was removed effectively by an anion-exchange bed when present as the metal oxyanion $HCrO_4^{-}$.

Semmens (1975) has observed very good removal of barium and lead by clinoptilolite, a cation-exchange resin. Very good removal of barium, methylmercury, and inorganic mercury have been reported by Logsdon and Symons (1973). Bowers (1971) noted the efficient removal of magnesium by ion exchange.

Although ion-exchange treatment can be designed for the removal of iron, manganese, and aluminum, the presence of these metals in water may impair the exchange capacity of a resin designed for the removal of other metals. Nordell (1961) stated that some removal of dissolved silica with a strongly basic anion resin is possible.

Table V-10
Removal of Metals by Water Treatment Processes

	Chemical Coagulation		Lime Softening				
Metal	Alum	Ferric Salts	Low pH 7.5-10	Excess pH 10.5-11.5	Activated Carbon	Reverse Osmosis	Ion Exchange
Ba	Poor(1)	Fair(1)	Good(1)	Good-V Good(1)	Poor(1)		V Good(5,6) Cation
Cd	Poor-Fair(1)	Good-V Good(1) pH >8.5	V Good(1)	V Good(1,2)	V Good(2)	V Good(5)	V Good(7) Cation
Cr	Poor(9)	Poor(9)		Poor(9)	V Good(2)	V Good(5)	V Good(7) Anion
Cu	Poor(1,3) pH 6.5-7	Fair(3)	Good(3)		Good(12)		
[illegible]	Good(3) pH 6.5-7					V Good(5)	
Pb	Good(3) pH 6.5-7		V Good(7)	Poor-Fair(11)	Good(12)		V Good(7) Cation
Hg			Poor-Fair(8)	V Good(8)	V Good(5)		V Good(7) Cation
Mn		Fair-Good(13) high pH		Good(13)			Fouls Resins(7)
Methyl Hg	Poor(1)	Poor(1)	Poor(1)	Poor(1)	V Good(1,3)		V Good(6) Cation
Inorganic Hg	Poor(1)	Fair(1)	Fair(1)	Good(1)	Good(1)		V Good(5)
[illegible]	Poor(9)	Poor(7)					
Ni	Poor(3) pH 6.5-7		Good(3)		Fair(12)	V Good(5)	
Ag				V Good(2)	V Good(2)	V Good(5)	Good(7) Cation
[illegible]					V Good(12)		
V					Fair-Good(12)		
Zn	Poor(3) pH 6.5-7		Good(3)		Poor-Fair(12)		

Removal Efficiencies

Poor : 0-20 percent Good : 70-90 percent
Fair : 20-70 percent V Good : >90 percent

References

1. Symons et al., 1975
2. Linstedt et al., 1971
3. [illegible], 1971
4. [illegible], 1974
5. Logsdon and Symons, 1975
6. [illegible], 1975
7. [illegible], 1971
8. [illegible], 1971
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12. [illegible], 1972
13. Logsdon and Symons, 1973

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Analysis of Drinking Water for Trace Metals

The literature on chemical analyses of trace metals in natural fresh water is voluminous. Only the most pertinent publications will be discussed here.

Brown *et al.* (1970) have prepared a comprehensive manual that contains methods used by the U.S. Geological Survey to collect, preserve, and analyze water samples for dissolved mineral and gas content. Among the topics discussed are the selection of sampling sites, frequency of sampling, sampling equipment, sample preservation, laboratory equipment and techniques, accuracy and precision of the analysis, and reporting of results. The methods of analysis are applicable to a wide range of water, from that with trace concentrations of dissolved metals to that with high concentrations.

The National Environmental Research Center of the EPA at Cincinnati, Ohio, has published methods of chemical analysis of water and wastes (U.S. EPA, 1971). The atomic absorption method is suggested for the determination of aluminum, cadmium, chromium, copper, lead, magnesium, manganese, silver, and zinc; and the flameless atomic-absorption method is suggested for mercury. Method selection was based on the following criteria:

1. The method should measure the desired constituent with precision and accuracy sufficient to meet data needs in the presence of the interferences normally encountered in polluted water.
2. The method should utilize the equipment and skills normally available in the typical water-pollution control laboratory.
3. The method should be in use in many laboratories or have been sufficiently tested to establish its validity.
4. The method should be sufficiently rapid to permit routine use for the examination of a large number of samples.

Guidelines establishing test procedures for analysis of various pollutants in water were published in the *Federal Register* on October 16, 1973. They included references to 71 test procedures for measurement of pollutants for which limitations were specified under the Federal Water Pollution Control Act Amendments of 1972.

Several professional associations have recommended procedures for analysis of water samples for various trace metals. Such publications are *Standard Methods for the Examination of Water and Wastewater* (Amer. Public Health Assoc., 1976), and *Annual Book of ASTM Standards* (Amer. Soc. for Testing Materials, 1970).

In addition, many symposium volumes and handbooks have summarized the state of the art for the analysis of trace metals in aqueous solution. These reviews can be found in Hume (1967), Boettner and Grunder (1968), Hemphill (1973), Cosgrove and Bracco (1973), and others.

Recent developments dealing with analytic methods for trace metals in waters are reviewed biannually in *Analytical Chemistry* and annually in *Journal of Water Pollution Control Federation* (Minear, 1975).

Sample Treatment. For the determination of trace metals in fresh water, large volumes of sample are required. Caution must be exercised in the proper collection and treatment of water samples, if the analytic results are to reflect the actual conditions of the water sampled. Water samplers sometimes introduce serious contamination. In selecting sample containers, care must be taken to avoid containers whose interior surfaces contain active metal-binding sites or that may release contaminating metals into the water sample. Inert plastic containers are usually preferred to glass. Polyethylene bottles are generally satisfactory. Ediger (1973) recommended a cleaning procedure of soaking containers in a 2% nitric acid solution for 24 h and then rinsing several times with metal-free water.

Rapid changes may occur in the chemical composition of water samples during storage, owing either to the introduction of contaminants from the containers or to selective adsorption of metals onto the walls of containers. Trace metals in water are also subject to change because of biologic activity. Water samples are usually stabilized by the addition of dilute acid. The EPA recommends the addition of 3 ml of 50% nitric acid to each liter of filtered sample. For unfiltered samples, 5 ml of concentrated nitric acid is recommended. Other preservatives have been recommended for metals known to be unstable in aqueous solution, such as silver (West *et al.*, 1967) and mercury (Omang, 1971).

Analysis. A general requirement in analytic chemistry is standardization of methods. Some methods that serve today as the legal standards in drinking-water quality control for trace metals are not sufficiently sensitive and accurate. The establishment of a method, moreover, does not guarantee that it will produce the same results when used by different analysts in different laboratories. A strong effort is required in evaluating and improving the analytic methods used in drinking-water quality control.

The analysis of trace metals is intimately related to the setting and enforcement of drinking water standards. The reliability and detectability of analytic methods may be the limiting factors in defining standards and maintaining surveillance. Methods must be reliable and provide a measurement of the species under consideration. Furthermore, in the case of effective monitoring programs, methods must be rapid and must have a reasonable cost.

In any trace analytic method, the first consideration is sensitivity. Because of the very low concentrations of some trace metals in natural water, methods should have sensitivities of a nanogram or less. Such methods may involve concentrating the sample; this should be avoided if possible. The stated sensitivity value for a particular method is generally not an exact figure. "Sensitivity" is sometimes defined as the concentration that yields a reading of 1% of full scale of the instrument; it is used in this manner in atomic absorption. The detection limits are usually defined as twice the background.

The specificity of an analytic method indicates the degree to which the method detects one element with no interferences from other elements that are present. Ideally, one would like methods that are specific for each element to be analyzed with few or no interferences.

Accuracy and precision of the procedures are important, but results will be less accurate and less precise as concentrations move into the micrograms-per-liter region. Each procedure should be checked for precision on real samples, and the data reported with respect to the standard deviation.

In selecting a method of water analysis for trace metals, sensitivity, speed, ease of operation, and relative lack of chemical interferences make the conventional and flameless atomic-absorption spectrophotometers instruments of choice. All analytic procedures recommended by the EPA and the U.S. Geological Survey for determining trace metals in water samples are based on atomic-absorption spectrophotometry. These instruments are generally available in analytic laboratories.

For general information on atomic-absorption analysis, books by Elwell and Gidley (1966), Slavin (1968), Ramirez-Munoz (1968), L'vov (1970), Price (1972), Reynolds and Aldous (1970), Kirkbridge and Sargent (1974), and Robinson (1975) are recommended.

Barium

Occurrence. Barium, one of the alkaline earth metals, occurs naturally in almost all (99.4%) surface waters examined, in concentrations of 2-340 μg/liter, with an average of 43 μg/liter (Kopp and Kroner, 1967). The drainage basins with low mean concentration of barium (15 μg/liter) occur in the western Great Lakes, and the highest mean concentration of 90 μg/liter is in the southwestern drainage basins of the lower Mississippi Valley. Finished water of public systems frequently (99.7% of supplies examined) contains barium, at 1-172 μg/liter, with a mean of 28.6 μg/liter. The 100 largest cities (Durfor, 1964) of the United States had a median concentration of 43 μg/liter, with a maximum of 380 μg/liter, but, 94% of all determinations were less than 100 μg/liter. Drinking water at the tap, as determined in 2,595 samples, had a maximum of 1,550 μg/liter; the maximum was found in one of only two samples that exceeded the interim standard of 1,000 μg/liter (McCabe, 1970).

Chemical Characteristics. Barium is slightly rarer than strontium in the earth's crust. It may replace potassium in some of the igneous rock minerals, especially feldspar. Barium sulfate (barite) is a common barium mineral of secondary origin. In stream water and most ground water, only traces of the element are present.

The reason for the small amount of barium in solution is the low solubility of barium sulfate. Because natural water usually contains sulfate, only trace amounts of barium will dissolve. Barium sulfate is soluble in pure water at 20°C--barium at 1.6 mg/liter and sulfate at 1.1 mg/liter. The solubility of barium sulfate increases considerably in the presence of chloride and other anions. However, water containing sulfate at more than a few parts per million will not carry barium at more than a few parts per million (USGS, 1959).

Metabolism. The metabolism of barium has been traced by radioisotope techniques and shown to be similar to that of calcium (Seaber, 1933; Bauer *et al.*, 1956). The digestive system is extremely permeable to barium, allowing for rapid transfer to and from the bloodstream (Bauer, 1957). The metal is transported in the plasma and disappears from the blood completely within 24 h.

Excretion of barium is different from that of calcium, in that the rate is greater in feces than in urine. In feces, 20% of barium is excreted in 24 h compared with 6% of calcium; in urine 7% of barium is excreted in 24 h, compared with 0.9% of calcium.

Health Effects. No vital metabolic function has yet been found for barium, although it is believed to be beneficial for rats and guinea pigs under specific dietary conditions (Underwood, 1971).

Barium is highly toxic when soluble salts are ingested. Fatalities have occurred from mistaken use of barium salt rodenticide. The fatal dose of barium chloride for man has been reported to be about 0.8-0.9 g, or 550-600 mg of barium (Sollman, 1957).

Industrial exposure to barium oxide and sulfate dusts produces a benign pneumoconiosis called "baritosis." Although barium poisoning is rare in industry, the potential from the more soluble forms is real. The American Conference of Governmental Industrial Hygienists set an airborn threshold limit value (TLV) for barium of 0.5 mg/m³. The limit was based on several years of observation of workers at Los Alamos exposed to barium nitrate.

Acute barium poisoning exerts a strong, prolonged stimulant action on all muscles, including cardiac and smooth muscle of the gastrointestinal tract and bladder. Barium is capable of causing nerve block (deNo 1946) and in small or moderate doses produces a transient increase in blood pressure by vasoconstriction (Gotsev, 1944).

There has been no determination of the chronic effects of barium administered repeatedly over a long period, either in food or drinking water.

Analysis. Conventional flame atomization does not have sufficient sensitivity to determine barium in most water samples; however, a barium detection limit of 10 µg/liter can be achieved, if a nitrous oxide flame is used. Renshaw et al. (1973) described a concentration procedure for barium that uses thenoyltrifluoroacetone-methylisobutylketone extraction at a pH of 6-8.

With a tantalum liner insert, the barium detection limit of the flameless atomic absorption procedure can be improved to 0.1 µg/kg (Renshaw, 1973).

Conclusions and Recommendations. A drinking-water guideline was derived from the 8-h weighted maximum allowable concentration (TLV) in industrial air of 0.5 mg/m³ set by the American Conference of Governmental Industrial Hygienists. It was assumed that, with an 8-h inhalation of 10 m³ of air, the daily intake would be 5 mg of barium, of which 75% was absorbed in the bloodstream and 90% transferred across the gastrointestinal tract. Based on the above assumptions, it was reasoned that a concentration of about 2 mg/liter of water would be safe for adults. To provide added safety for more susceptible members of the population, such as children, a level of 1 mg/liter was recommended (Stockinger, 1958). There have been no long-range feeding studies to confirm the safety of this barium intake. The limit set in the U.S.S.R. is 4 mg/liter of water. International and European standards do not list barium upper limits, because available information is insufficient.

It is rare to find sources of water that exceed a barium concentration of 1 mg/liter, although a concentration of 1.55 mg/liter has been recorded in drinking water. The 1975 Analysis of Interstate Carrier Water Supply Systems showed none exceeding the 1 mg/liter standard, but, seven systems in eastern Pennsylvania, three in Kentucky, and one in New Mexico were at the upper limit. In view of the number of persons exposed, it would be desirable to study any risk that might be associated with chronic ingestion of barium.

Animal studies should be undertaken at least, to determine the toxic effects of long-term ingestion of barium at low concentrations.

Beryllium

Occurrence. A relatively rare element, found chiefly in the mineral beryl (beryllium aluminum silicate), beryllium is not likely to occur in natural water in appreciable concentrations. Although the chloride and nitrate are very soluble and the sulfate moderately so, the carbonate and hydroxide are almost insoluble in cold water (McKee and Wolf, 1963).

Beryllium is used primarily in metallurgy to produce special alloys, in the manufacture of x-ray diffraction tubes and electrodes for neon signs, and in nuclear reactors (Browning, 1961). It is also used in rockets and in missile fuels. Crailey (1972) presented an extensive discussion of the many modern uses of beryllium metal, beryllium-copper alloys, beryllium oxide, and minor beryllium compounds. The consumption of beryl increased from 1,200 short tons in 1961 to 8,465 tons in 1969.

Using emission spectroscopy, Durum and Haffty (1961) measured beryllium in 59 samples of surface water from 15 rivers in the United States and Canada. The highest concentration observed was less than 0.22 µg/liter. Kopp and Kroner (1967) noted the presence of beryllium in 85% of their samples from the 15 major river basins of the conterminous United States; the concentration ranged from 0.01-1.22 µg/liter, with an average of 0.19 µg/liter. According to Standard Methods (APHA, 1976), beryllium has been reported to occur in U.S. drinking water at 0.01-0.7 µg/liter, with a mean of 0.013 µg/liter.

In a study of many groundwater samples from the western United States, beryllium was detected in only three highly acid mine waters. Beryllium discharged to ground water will not travel far in neutral solution, because it is rapidly adsorbed by the clay in the soil. In the eastern United States and in Siberia, surface water was reported to contain beryllium at 0.1-0.9 µg/liter. Pacific Ocean water contains 2-9 µg/liter (Griffitts et al., 1976). According to the NAS-NAE report on water-quality criteria (NAS, 1973), the concentration of beryllium in seawater is only 6×10^{-4} µg/liter.

Food does not appear to be a significant source of human exposure to beryllium. According to Griffitts et al. (1976), "there is no evidence at present that beryllium is moving from soils into food or feed plants in the United States in amounts that are detrimental to plants, animals, or people." Furthermore, "the forms of beryllium in plants and their digestibility by animals have not yet been determined."

Chemical Characteristics. Although beryllium is in the same group of elements as the alkaline earth metals, it shares few properties with them. Beryllium replaces silica in the structure of some igneous rock minerals and is present as independent beryllium minerals in pegmatites, the most important of which is beryl. In the weathering process, beryllium (like aluminum) is concentrated in hydrolyzates and does not go into solution to any appreciable degree. Beryllium is not likely to be found in natural water in greater than trace amounts, because of the relative insolubility of beryllium oxides and hydroxides at the normal pH range of such water. The solubility of the oxide is reported as about 0.02-0.07 ppm in pure water at about 20°C. The sulfate and chloride of beryllium are very soluble, but would hydrolyze and lower the pH. In the presence of sodium hydroxide (high pH), beryllium hydroxide is soluble, probably because of the formation of anion complexes. The effects of other ions or cations on the solubility of beryllium are not known (USGS, 1959).

Some data on adsorption of trace quantities of beryllium in water by glass and plastic containers have been reported. At a pH of 1.5, there was no adsorption of beryllium by the container. However, at a pH of 7 and 8, there was considerable adsorption. Adsorption of beryllium by naturally occurring minerals is probably an important cause of the low concentrations in water, inasmuch as such adsorption seems to proceed effectively at pH values common in natural water.

Metabolism. Absorption of beryllium from the digestive tract is slight (about 0.006% of that ingested), and excretion is fairly rapid (Browning, 1961).

Health Effects. In a comprehensive review, Pomelee (1953) reported that there was no indication that beryllium in any form is harmful when taken orally.

Inhalation of particles is by far the major hazard to humans from this metal. Beryllium has been incriminated in pulmonary ailments of workers exposed to beryllium dusts (Browning, 1961). Since the development, in about 1947, of spectrochemical techniques for detecting beryllium in air, there has been a substantial increase in the number of reported cases of beryllium poisoning.

No information was uncovered to indicate that beryllium is a beneficial or necessary component of human nutrition.

Rats were healthy after 2 years on a diet that included beryllium sulfate at about 6.6 mg/day, equivalent to beryllium at 1.0 mg/kg of body weight per day. Four dogs showed no ill effects after 19 months of daily ingestion of beryllium sulfate at 10 mg/kg of body weight; 1 dog lost weight after 9-months and was killed for examination. No evidence of tissue damage was found (Pomelee, 1953). When mice were fed beryllium at 5.0 mg/liter in drinking water for life, slight effects on the body weight of females (but not males) were disclosed, and there were no effects on the life span and survival of either sex. These studies with mice indicated that beryllium is poorly absorbed through the gut and that ingestion is not a hazard (Schroeder and Mitchener, 1975). According to Stokinger (1972), the dietary LD_{50} of beryllium sulfate in rats after 175 days was 2,750 mg/kg of body weight per day. The beryllium metal in beryllium oxide eaten in the diet at 5.0% is so poorly absorbed that no effect on growth occurred over long periods of feeding. Beryllium sulfate did not interfere with growth until a concentration of 1.4% (14,000 mg/kg of diet) was reached.

With the data for 10 of the 15 river basins studied by Kopp and Kroner (1967), Berg and Burbank (1972) attempted to establish correlations between carcinogenic trace metals in water supplies and cancer mortality--8 metals compared with 34 types of cancer, for a total of 272 comparisons. At the 0.05 level of significance, they expected about 14 comparisons to show positive correlations. In fact, 28 positive correlations were found, 5 of which were associated with beryllium. When they studied these findings in further detail, however, especially with respect to bone cancer, the 5 correlations were not meaningful. Berg and Burbank concluded that the correlations were not consistent with a waterborne pattern and could be explained by other known factors.

The inclusion of beryllium in the work of Berg and Burbank (1972) was prompted by the fact that beryllium was the first metal to produce cancers in animals with any substantial frequency away from the site of administration. Stokinger (1972) noted that soluble beryllium sulfate is about equally toxic (in milligrams per kilogram of body weight) to rats, mice, dogs, monkeys, and rabbits, whether administered by inhalation, intratracheally, intravenously, or subcutaneously. When beryllium is transported via the bloodstream from its initial site of deposition, a significant part of the administered dose ends up in the skeleton, irrespective of the mode of administration. In the bones of animals, it has been shown to produce osteosarcoma; but this has been demonstrated only in animals and not yet in humans who have beryllium lung disease (pulmonary sarcoidosis) from inhaling beryllium dust (IARC, 1973).

According to Sterner and Eisenbud (1951), acute pneumonitis among human beings has been caused by exposures to beryllium in the atmosphere at concentrations of less than 1.0 to over 100 $\mu g/m^3$ of air. The symptoms of beryliosis include skin and lung diseases of variable severity. The reactions of people to a given exposure are said to vary widely, but apparently any person will show a reaction if time and degree of exposure are great enough.

It became apparent by 1947 that many cases of what was then thought to be pulmonary sarcoidosis were appearing among beryllium production workers as a result of inhalation of beryllium compounds and metallic dust. On the recommendations of an *ad hoc* advisory committee, the Atomic Energy Commission (AEC) established strict in-plant limits for beryllium in the atmosphere and much stricter limits for neighborhoods near AEC plants (Stokinger, 1972). Apparently, beryliosis is confined to the lungs, and beryllium is not translocated to other parts of the body. Any sputum that might be swallowed would get into the digestive tract, where beryllium has been shown to be relatively harmless. No unusual incidence of lung cancer has yet been found among workers exposed to beryllium, although sizable numbers had exposures more than 20 years ago. This experience indicates that, if beryllium proves to be carcinogenic in humans, it is of low potency (Stokinger, 1972).

Analysis. According to *Standard Methods* (USEPA, 1976), atomic-absorption spectrophotometry and colorimetry are equally suitable for the determination of beryllium. Direct flame atomization offers a detection limit of 2 μg/liter. Sachdev and West (1969) have described a concentration procedure that uses solvent extraction with an oxine-acetylacetone-dithizone combination at a pH of 6.0. The detection limit can be lowered to 0.03 μg/liter when the graphite furnace is used for atomization. Chapman *et al.* (1974) have used flameless atomic absorption for beryllium analysis.

Conclusions And Recommendations. Beryllium is relatively harmless when ingested in food and water, except at very large continuing dosages. It is present in natural surface water at concentrations generally less than 1.0 μg/liter, with averages of less than 0.2 μg/liter; hence, it presents no hazard in drinking water. The U.S.S.R. has set a limit of 0.2 μg/liter, but the World Health Organization has not established any limit (Stoefen, 1973). The EPA has not promulgated any limit for beryllium in its *National Interim Primary Drinking Water Regulations* (1975). Beryllium is known to cause cancer in various species of laboratory animals, but to date has not been associated with human cancer. Because of the strong association of beryllium with cancer in animals a continuing effort should be made to study both through epidemiology and chronic low level feeding studies the toxicology of beryllium.

Cadmium

The sources, distribution, metabolism, and toxicology of cadmium have been reviewed by Friberg *et al.* (1971, 1974, 1975), Underwood (1971), Nordberg, (1976), and Copenhaver *et al.* (1973).

Occurrence. The principal industrial uses of cadmium are in electroplating, in pigment manufacture, and as a plasticizer, chiefly in polyvinylchloride. Cadmium occurs in zinc ores and is an important byproduct in the metallurgy of zinc. Because cadmium is an impurity in zinc, cadmium should possibly receive some consideration when poor grades of zinc are used for galvanizing. The use of cadmium-plated containers in food- and beverage-handling materials is now prohibited by the Food and Drug Administration because acute cadmium poisoning has been recognized in man after consumption of food and particularly acidic beverages stored in cadmium-plated containers. Except where stated, estimates of intake and critical renal concentration are taken from *Cadmium in the Environment*, II and III (1974, 1975).

In streams and rivers, the concentration of cadmium tends to be higher in sediment than in filtered running water. From studies in Japan (Friberg, 1974) and upstate New York (Kubota *et al.*, 1974; Durum, 1974), it appears that most fresh water contains cadmium at less than 1 μg/liter. The U.S. Geological Survey reported that about 46% of samples contained detectable amounts--1 μg/liter or more. Regional differences are noted within the United States, with the higher concentrations found in runoff water in the Northeast, in some urbanized areas in the South, and in the central states. This distribution pattern suggested to Durum that pollution sources and rainfall may be the major contributors of cadmium in river water.

Carbonate content and pH influence the stability and solubility of cadmium in water. It is least soluble at a pH of approximately 8-9 and becomes increasingly soluble as the pH decreases. But, the median concentration in surface water in most areas is less than the detection limit (<1 μg/liter of water). Durum used filtered samples and found that 4% of surface waters in the United States exceeded the 1962 U.S.P.H.S. drinking-water standard of 10 μg/liter. However, the U.S.P.H.S. National Community Water Supply study indicated that the drinking-water standard for cadmium was exceeded by only 0.1% of 969 water-supply systems tested, which served an estimated 18 million people. Craun and McCabe (1975) reported data on the interaction between soft water and accumulation of cadmium in the distribution systems for Boston and Seattle. This survey indicated that 13% of samples obtained in Boston showed a higher concentration at the tap than at the treatment plant. In

Seattle, which has more acidic water, 51% of the sample showed an increase. Both running and standing samples were obtained. In Seattle, 7% of the samples exceeded the 10 μg/liter drinking-water standard; in the Boston area, none exceeded this standard.

There is a wide consensus that the cadmium content of food is the major source of cadmium for the general population. Friberg *et al.* (1974) estimated that the average daily intake for adults is approximately 50 μg. If this estimate is adjusted on a caloric basis for children consuming a similar diet, the intake at 2-3 years of age would be about one-third to one-half of the adult intake. There is a rather wide range in the estimates of cadmium intake in food. This may be due largely to difficulties in the measurement of trace amounts of cadmium. Because cadmium is a contaminant of superphosphate fertilizers and because of current plans to use sewage sludge for agricultural purposes, it is the consensus of most experts that the food supply should be carefully monitored for cadmium and other trace metals.

Although air cadmium concentrations may be high near lead, zinc, and cadmium smelters and refineries, it is generally about 1 ng/m^3 elsewhere.

Cigarette tobacco contains cadmium at about 1 ppm. Friberg (1974) has estimated that the smoking of 1 pack of cigarettes a day can contribute 2-4 μg of cadmium a day.

The best-described accident related to discharge of cadmium into water is the occurrence of Itai-Itai disease among residents along the Jintsu River in Japan (Friberg et al., 1971). These residents were apparently exposed not only through the drinking of water, but also through the ingestion of rice grown in the contaminated water.

Chemical Characteristics. Elemental cadmium is present in rocks in much lower quantities than those reported for zinc. Only traces are likely to be found in natural water, but cadmium may be introduced in amounts significant from a health standpoint by solution from containers or tubing or by waste disposal.

Cadmium probably could be present only in small amounts in water with the normal alkaline pH, because of the low solubility of the carbonate and hydroxide. Cadmium hydroxide is soluble at about 1 mg/liter at 25°C. Exact data regarding solubility of the carbonate are not available. At a pH below about 4.5, the solubility of cadmium would be controlled by other factors and would probably be greater (USGS, 1959).

Metabolism. The total daily intake of cadmium from air, water, food, and cigarettes is estimated to range between 40 μg/day (for nonsmoking rural residents who have negligible air exposure and consume a low-cadmium diet) and 190 μg/day (for smokers living in industrialized cities and consuming a high cadmium diet).

Absorption from the digestive tract is thought to average about 10%. However, a number of factors, including dietary calcium and protein and age, may have an important bearing on this. For the digestive-tract route of assimilation, the major organs of cadmium storage are the liver and renal cortex. The renal cortex may contain one-third of the total cadmium body burden. The biologic half-life of cadmium in these organs is variously estimated at 13-38 years. Urinary excretion is low, from 1-9 μg/day. Because cadmium tends to accumulate, a more useful way of looking at the question is to consider the rate of accumulation. The human placenta is apparently highly impermeable to cadmium. The total body burden is estimated at 1 mg at birth and at 15-50 mg at the age of 50 years. This is consistent with an average accumulation of 0.9-1.8 μg/day. There is a major need for a more reliable estimate of the rate of cadmium accumulation.

The renal cortex is considered to be the critical organ for accumulation of cadmium from low level dietary exposures, and the critical concentration for renal cortex is approximately 200 μg/g of tissue (wet weight) (Friberg et al., 1974; Nordberg, 1976). At greater concentrations, irreversible renal injury may occur. In the outbreak of itai-itai disease on the Jintsu River, renal cortical cadmium concentration was estimated at 600-1,000 μg/g of tissue (wet weight) in those most severely (and irreversibly) affected. With an assumed water consumption of 1.5 liters/day, the average cadmium intake from water was estimated at 5 μg/day, or less than 10% of the total intake.

Health Effects. In industry, after overexposure to cadmium at high concentrations (50 $\mu g/m^3$) well in excess of that for the general population, bronchitis, emphysema, anemia, and renal stones have been found. Among the general population, gastrointestinal upsets similar to "food poisoning" have been reported in association with consumption of food or beverages conveyed in cadmium-plated vessels. Sporadic outbreaks of this sort occur when cadmium-plated vessels not intended for food are used to prepare lemonade and other acidic beverages for picnics and similar outings.

For the general population, the major route of absorption is through the gastrointestinal tract. The major effects are likely to be on the kidney. There is an extensive literature--reviewed by Friberg (1971, 1974, 1975), Nordberg (1976), and Sandstead (1974)--on this problem. Experimental data indicate that the zinc:cadmium ratio in the organs is an important determinant of cadmium toxicity (in most foodstuffs, the dietary ratio of cadmium to zinc is 1:100; it is highest in meat products and lowest in dairy products), and there is some evidence that the intake of sodium may also influence cadmium toxicity. There are no dose-response data. Limited autopsy data suggest that average renal cortical concentrations of cadmium in American and European populations are generally less than 50 µg/g of tissue (wet weight)--less that the projected critical concentration by a factor of 4 or more.

In addition to the suspected interactions between cadmium, zinc, and calcium, recent experimental studies indicate that cadmium at very high doses can interfere with the activation of vitamin D in both liver and kidneys to the final active 1,25-dihydroxycholecalciferol (Nordberg, 1976; Sandstead, 1974). There is also evidence from animal studies that cadmium is implicated in the etiology of hypertension (Schroeder, 1965); the thresholds and dose-response relationships are unknown. There is some evidence that cadmium is carcinogenic in the rat, but no substantial evidence to implicate it with human cancer (IARC, 1973). Cadmium is known to be teratogenic in the rat following rather high (2-13 mg/kg) doses on specific days of gestation (Chernoff, 1973).

Another effect observed--at high doses in rats--is ease of producing testicular and ovarian necrosis when cadmium is given by injection. This same effect can be seen in rats (who can not vomit) with high oral doses.

There are no identified hypersusceptible segments of the human population. Although victims of Itai-Itai disease were predominantly multigravid, postmenopausal women, this does not mean that these alone are predisposing conditions. It should, however, be noted that, on a body weight basis, infants may have a higher intake of cadmium. If, in fact, calcium intake is an important protective factor, it is well to note that a significant proportion of the population from school age up is lactose-intolerant and may voluntarily reduce milk intake and hence calcium intake on this account. If, as appears likely from experimental studies, zinc is an important protective factor against cadmium toxicity, it is worth noting that preliminary evidence indicates that those with hemoglobin SS or SC have shown signs of zinc deficiency. Further studies in these groups appear warranted.

Analysis. Direct flame atomization has a cadmium detection limit of 7 µg/liter. Most reported analyses, however, involve some form of concentration. The U.S. Geological Survey procedure (Brown *et al.*, 1970) recommends extracting the cadmium as an ammonium pyrrolidine dithiocarbamate at a pH of 2.8 with methylisobutylketone. Other concentration procedures have been described for fresh water (U.S. EPA, 1971; Traversy, 1971; Kaminski, 1974; Kinrade and Van Loon, 1974; Kubota *et al.*, 1974; Korkisch and Sorio, 1975; Aldous *et al.*, 1975).

The sampling boat and Delves cup techniques have cadmium detection limits of 0.1 and 0.05 μg/liter, respectively. Using the graphite furnace to atomize the sample can improve detection to 0.005 μg/liter. Paus (1971) has used the graphite furnace to determine cadmium in lake water at concentrations of 0.5-2.1 μg/liter. Other methods have been reported by Dolinsek and Stupar (1973), Surles *et al.* (1975), and Rattonetti (1974). Barnard and Fishman (1973) have critically evaluated the use of the graphite furnace for cadmium determinations in fresh water.

For all types of biologic samples, the available data indicate that either background correction or extraction is essential when determinations are made by atomic-absorption spectrophotometry, owing to the enhancing effect of sodium on the cadmium signal. Although it has not been fully explored, it appears that electrochemical techniques may be more suitable, although somewhat less sensitive, because such measurements may be influenced, to a lesser degree, by matrix effects. Except on a large-group basis, it appears that the measurement of cadmium in blood and spontaneously voided urine is of relatively little value, because they are not reliable indicators of the concentrations of cadmium in the organs, particularly in the renal cortex.

For the reasons stated above, one should scrutinize data carefully. It is likely that data obtained during the early years of atomic-absorption spectrophotometry are not reliable, because the background effect, particularly of sodium, was not appreciated at the time.

Conclusions and Recommendations. There should be a comparison of the intakes of cadmium in various industrial and geographic regions and an attempt to correlate them with specific diseases. These kinds of correlations should also be done on autopsy samples. There is a need to analyze, particularly in soft-water areas, the accumulation of cadmium in drinking water at the tap. There is also a need for certified reference samples, such as the NBS bovine liver and orchard leaves. Interlaboratory comparisons, exchange of standards, and establishment of a reference method are also warranted. The possible effect of cadmium on vitamin D metabolism needs investigation. The available data do not suggest any need to change the present drinking-water standard of 10 μg/liter, although there is a clear need for data on soft aggressive water areas.

Chromium

The NAS-NRC has recently (1974) completed an extensive review of the medical and biologic effects of chromium, which has been reviewed and excerpted for this report. Additional material has also been included when necessary.

Occurrence. Durum and Haffty (1961) reported a range of concentrations for chromium in U.S. rivers of 0.7-84 µg/liter. Kopp and Kroner (1967) detected chromium in 24.5% of the samples examined, with concentrations ranging from 1-112 µg/liter and averaging 9.7 µg/liter. In a study of surface and groundwater in Canada, all but two of 240 samples examined were below 50 µg/liter (Procter & Gamble, 1974). In 1974, a maximum dissolved chromium concentration of 30 µg/liter was recorded in water from the Pecos River, New Mexico; the Los Angeles River; and the Columbia River, Oregon (USGS, 1974). In a 1970 survey, 11 of 700 samples had chromium concentrations of 6-50 µg/liter, with none exceeding 50 µg/liter (Durum et al., 1971). Ackermann (1971) reported chromium concentrations below 5 µg/liter for 18 of 37 river stations in Illinois; the maximum was 50 µg/liter.

Chemical Characteristics. Elemental chromium is amphoteric and can exist in water in several different states. It is present in minor amounts in igneous rocks and is much more abundant in basic and ultrabasic types than in the more silicic types of rocks. In attack by weathering, chromium in cationic form (CrIII) behaves somewhat like iron and is largely retained in resistates and hydrolysates. Very little chromium goes into solution. Natural water therefore would be expected to contain only traces of chromium as a cation, unless the pH were very low.

Chromium, under strongly oxidizing conditions, may be converted to the hexavalent state and occur as chromate anions. Natural chromates are rare and, when CrO_4^{-2} is present in water, it is usually the result of pollution by industrial wastes. Fairly high concentrations of chromate anions are possible in water with normal pH (USGS, 1959).

A study by Schroeder and Lee (1975) indicated that the oxidation state of chromium may be altered in natural water. Because of the possibilities for oxidation of Cr (III) and reduction of Cr (VI), they concluded that water-quality standards should be based on total chromium, rather than on hexavalent chromium.

Metabolism. Because of analytic problems related to the determination of chromium, data on the absorption and metabolism of chromium must be interpreted with caution. Trivalent chromium affects glucose metabolism, binds strongly with plasma albumin, and interacts with manganese in glucose metabolism (Hambridge, 1971).

In a study in which rats were administered trivalent chromium for a short time, there was no glucose metabolism effect, whereas administration for 15-120 days markedly affected glucose metabolism. Oversupply is reported to be no problem, and there appears to be a homeostatic mechanism for trivalent chromium involving a hepatic or intestinal transport system that rejects excessive accumulation.

Oral ascorbic acid converts hexavalent chromium to trivalent chromium and, when given within an hour or two, reduces gastrointestinal tract injury from hexavalent chromium (Hambidge, 1971).

Glucose tolerance in man declines with age; as many as two-thirds of an elderly population sampled in the United States had an abnormal glucose tolerance test. Tissue chromium in the United States also declines with age (Streeten _et al_., 1965).

The average daily intake of chromium in the United States varies widely due to diet and geography. Estimates range from 5 - 115 μ/day with an average of 60 - 65 μg/day (NAS, 1974) to 5 - 500 μg/day with an average of 280 μg/day (Schroeder, 1970).

It has been reported that, regardless of dietary history or amount administered, only 0.5-3% of a given dose of trivalent chromium is available to the organism. The degree of absorption depends on the chemical form of the chromium and ranges from 0.1-1.2% of trivalent chromium salts to about 25% of the glucose tolerance factor.

Chromium is excreted in urine and feces with the urinary pathway accounting for 80%. Nearly all chromium in urine is present in the form of low-molecular-weight complexes; very little protein bound chromium is excreted. Estimates of excretion also vary between wide extremes. In two carefully controlled studies, averages of 5 and 4 ug/liter were found. Mean 24 h urinary chromium excretion of 20 young adults was 8.4 ug with a range of 1.6 - 21 ug (NAS, 1974). Other estimates have shown an average urinary excretion from 3 - 160 μg/day with an average of 138 μg/day (Schroeder, 1970).

It is possible that pH plays a role in the physiologic distribution of hexavalent and trivalent chromium; the trivalent precipitates at physiologic pH and forms chromic hydroxide. Trivalent chromium may also precipitate with proteins (IARC, 1973).

According to Schroeder (1974), the background body burden of chromium in Americans is low and declines between the ages of 14 and 44. Body burdens of Africans, Near Easterners, and Orientals are much higher. Thais had higher tissue chromium concentrations than any other group.

Organ distribution studies have been inconclusive. Results in rats given doses of chromium chloride showed the ovaries and spleen to have the highest uptake and, the kidneys and liver the next highest, with the lungs, heart, pancreas, and brain lower. But when chromium was given in the form of glucose tolerance factor, the results were different; the liver accumulated most, followed by the uterus, kidneys, and bone. In autopsies of humans, the highest accumulation has been in lungs; this suggests that humans are accumulating most chromium from the air, rather than from water or food (NAS, 1974).

Health Effects. Acute systemic poisoning from chromium may result from accidental exposures, from therapeutic uses of chromium, and from suicide attempts. Principal damage to the body is tubular necrosis of the kidney. Cats fed 50-1,000 mg/day of chromic phosphate for 80 days were not affected. Rats fed trivalent chromium at 25 ppm for 1-year or 5 ppm for a lifetime were also not affected (NAS, 1974).

> Hexavalent chromium chemicals can be tolerated by animals in low concentrations, especially when they are administered in feed or drinking water, in which the degree of absorption is a factor. For example, rats tolerated hexavalent chromium in drinking water at 25 ppm for a year, and dogs showed no effect of chromium as potassium chromate at 0.45-11.2 ppm over a 4-y period. Even higher concentrations have been reported by some investigators. However, larger doses of hexavalent chromium are highly toxic and may cause death, especially when injected intravenously, subcutaneously, or intragastrically (Pg 82, NAS, 1974).
>
> Chronic toxicity can be observed in several mammalian species with hexavalent chromium in the drinking water in concentrations of more than 5 mg/liter. At this concentration, the element was found to accumulate in rats, but it caused no changes in growth rate, food intake, or results of blood analysis. Even 25 mg/liter in the drinking water failed to produce changes in these characteristics or in the histologic appearance of the tissues after 6 months. Dogs tolerated hexavalent chromium in the water at up to 11.2 mg/liter for 4 years without ill effects. The minimal lethal dose in dogs is approximately 75 mg of chromium as sodium chromate, when injected intravenously. The salt causes acute hypertension, hypocholesterolemia, and hypoglycemia. Growing

chickens showed no detrimental symptoms when they were fed 100 μg/g in diet (Pg 29, NAS, 1974).

Although hexavalent chromium has long been recognized as a toxic substance, trivalent chromium is considered by most investigators to be relatively innocuous and even (in microgram amounts) essential to human health. Hexavalent chromium produces hemorrhage of the gastrointestinal tract after ingestion. Inhaled chromate may cause cancer of the respiratory tract in occupationally exposed individuals (IARC, 1973). It also produces ulceration on dermal exposure.

The chronic adverse effects most often considered in chromium toxicity are respiratory and dermatologic. "It now appears that investigators agree on several points:"

1. People who work with hexavalent chromium can develop cutaneous and nasal mucous-membrane ulcers, whereas exposure to trivalent chromium does not produce these effects.
2. People who work with hexavalent chromium compounds can develop contact dermatitis from these agents, and they react to patch and intracutaneous tests with nonirritant concentrations of potassium dichromate.
3. Hexavalent chromium in tissue is reduced to the trivalent form.
4. Hexavalent chromium has greater diffusibility and solubility in tissue than trivalent chromium.
5. Hexavalent chromium can readily penetrate membranes.
6. Trivalent chromium can readily bind with some proteins to form complexes (p. 72, NAS, 1974).

Atherosclerosis in relation to chromium has been of interest. Studies have indicated that atherosclerosis can be induced in animals by chromium-deficient diets (Hambridge, 1971).

With regard to carcinogenicity, intraosseous, intramuscular, subcutaneous, intrapleural, and intraperitoneal injections of chromium compounds have been reported to cause the development of sarcomas in rabbits, mice, and rats. There is some evidence that calcium chromate in the form of a pellet attached to the bronchial mucosa in rat lung may be carcinogenic, but, there is no support for the view that it constitutes a carcinogenic hazard in human food (Sunderman, 1971). An IARC working group (1973) concluded "there is no evidence that non-occupational exposure to chromium constitutes a cancer hazard."

Analysis.

Both hexavalent and total chromium concentrations are commonly determined in water. Direct aspiration of samples into a flame is used to determine the total chromium content with a detection limit of 3 µg/liter. The U.S. Geological Survey (Brown et al., 1970) has suggested a procedure for the determination of hexavalent chromium by extraction with ammonium pyrrolidine dithiocarbamate at a pH of 2.8. The same procedure is also used for the determination of total chromium after oxidation of any trivalent chromium present to the hexavalent state with potassium permanganate. Nix and Goodwin (1970) have used diethyldithiocarbamate for extraction of chromium.

Fernandez and Manning (1971), Barnard and Fishman (1973), and Suries et al. (1975) have used the graphite furnace to increase sample vaporization for the determination of total chromium; the detection limit is 0.1 µg/liter.

Conclusions and Recommendations. The NAS chromium report offered recommendations for research. These were among the most pressing:

1. At present, only two analytic techniques can be successfully used for accurate quantitative determination of chromium at the low concentrations that exist in many environmental media, especially in plant and animal tissue—neutron activation and shielded-arc emission spectrography. Both methods are expensive and time-consuming and require considerable experience and thus are not applicable to large-scale environmental studies. Laboratory research, using the latest analytic instrumentation, is needed for the development of sensitive, accurate, and precise methods for the analysis of chromium that could be used by most laboratory investigators....
2. Accurate background information on normal concentrations of chromium in various media is necessary for predicting trends.
3. The potential toxicity of chromium depends on its valence state. There are no techniques for estimating the concentration of chromium in relation to its valence state, especially in animal and plant tissue. Data of this type also would be extremely useful for understanding the biologic function and availability of chromium....
4. Research is needed to ascertain the relation between exposure to airborne chromium and chromium concentrations in urine, blood, and other biologic media, such as hair. If any relation is demonstrated, biologic standards for exposure may become possible. (pp. 112-113, NAS, 1974).

There is a strong link between airborne chromium and lung cancer, but there is no firm evidence to establish a relationship between non-occupational exposure by any other route. There is also evidence that chromium has an important role in maintaining glucose metabolism and may also be a factor in atherosclerosis. It is therefore possible that with the exception of occupational exposure a deficiency of chromium may be more of a problem.

The present interim drinking water standard of 0.05 mg/liter is less than the no-observed-adverse-health-effect level. Consideration should be given to setting the chromium limit in terms of the hexavalent form. Extensive work is urgently needed to establish the role of dietary chromium with regard to atherosclerosis and glucose metabolism as well as its possible carcinogenic effects at low levels in life time feeding studies.

Cobalt

Occurrence. Cobalt and its salts are used for making alloys, in nuclear technology, as pigments in the china and glass industry, and as binders in the tungsten-carbide tool industry. Cobalt may be divalent or trivalent. Solutions containing cobaltous ions (Co^{++}) are relatively stable, but cobaltic ions (Co^{+++}) are powerful oxidizing agents and are thus unstable in natural water. Cobaltous chloride, $CoCl_2$, is a highly soluble salt that is used in the manufacture of sympathetic ink, barometers, and hydrometers and in galvanoplating, ceramics, and (as a feed supplement) salt licks for ruminant animals. Cobaltous nitrate, $Co(NO_3)_2$, is used in the manufacture of cobalt pigments and sympathetic ink and in decorating porcelain. Cobaltous sulfate, $CoSO_4$, a red crystalline substance that is readily soluble in water, is used in decorating and plating and for remedying cobalt deficiencies in cattle and sheep (McKee and Wolf, 1963).

Durum et al. (1971) examined more than 720 river-water samples during low flows in October 1970 in the 50 states and Puerto Rico; 37% contained traces of cobalt, in the range of 1.0-5.0 μg/liter; 54% contained cobalt below the detection limit, i.e., less than 1.0 μg/liter; 21 samples (2.9%) contained 6-9 μg/liter; 20 samples (2.8%) contained 10-19 μg/liter; 17 samples (2.4%) contained 20-39 μg/liter and six samples (0.8%) contained 40-99 μg/liter.

In an earlier report, using emission spectroscopy on 59 samples of water from 15 rivers in the United States and Canada, Durum and Haffty (1961) found a maximum cobalt concentration of 5.8 μg/liter in the Mississippi River at Baton Rouge, Louisiana. Kopp and Kroner (1967) noted the presence of cobalt in only 2.8% of their samples from the 15 major river basins of the conterminous United States, with concentrations ranging from 1.0 to 48 μg/liter, and a mean of 17 μg/liter.

In Russia, Barabannik et al. (1961) measured cobalt in two artesian supplies used for drinking water at Kiev. These wells vary from 89.6-261.5 m in depth. In the shallower, cobalt concentrations varied from 0.61-2.41 μg/liter, with an average of 1.32 μg/liter; in the deeper, the range was 0.43-1.9 μg/liter, with an average of 0.94 μg/liter.

Green leafy vegetables are the richest and most variable sources of cobalt in human diets; dairy products and refined cereals are among the poorest. Typical concentrations in food are 0.4-0.6 mg/kg (dry weight) in spinach, 0.2 in cabbage and lettuce, 0.01 in cornseed, and 0.003 in white flour. These figures indicate that the diet contributes far greater amounts of cobalt than are ever likely to be obtained from water.

Chemical Characteristics. Cobalt and nickel are very similar in chemical behavior. Both are present in igneous rocks in small amounts and are more prevalent in the basic and ultrabasic types than in silicic rocks. In the process of weathering, cobalt may be taken into soluton more readily than nickel but is adsorbed to a great extent by the hydrolysate or oxidate sediments. Cobalt may be taken into solution in small amounts through bacteriological activity similar to that causing solution of manganese.

Metabolism. Cobalt is part of the vitamin B_{12} molecule and as such is an essential nutrient. Ruminants can synthesize their own vitamin B_{12} if they are given cobalt orally. A wide margin of safety (well over 100) exists between the required and toxic doses for sheep and cattle (NAS, 1973). Nonruminants, such as humans, require preformed vitamin B_{12}, in which the one cobalt atom per molecule accounts for only 4.34% of the total molecular weight of the vitamin. The requirement of humans for cobalt in the form of vitamin B_{12} is about 0.13 μg/day (U.S. FDA, 1975).

Health Effects. According to Underwood (1973), cobalt has a low order of toxicity in all species studied. Daily doses of 3 mg/kg of body weight (about 1,000 times normal) can be tolerated by sheep for many weeks without harmful effects.

Using the data on 10 of the 15 river basins studied by Kopp and Kroner (1967), Berg and Burbank (1972) examined correlations between potentially carcinogenic trace metals in water supplies and cancer mortality among humans. Cobalt showed no correlation with any of the 18 different types of cancer studied.

Cobalt has been severely indicted as a toxicant when added to beer to promote the formation of foam (U.S. FDA, 1975). Clusters of congestive heart failure deaths were observed in Quebec, Omaha, Nebraska and Leuven, Belgium, among heavy beer-drinkers about 1965. Cobalt salts had been added to the beer at cobalt concentrations of 1.2-1.5 mg/liter (Underwood, 1973). At such concentrations, the consumption of 24 pints a day would supply only about 15 mg of cobalt sulfate--well below the amount that can be taken with impunity by normal people. In fact, cobalt salts have been used therapeutically at up to 300 mg/day without cardiotoxic effects. The episodes were clearly attributed to the cobalt; the toxicity was no longer observed when the cobalt was removed. Research has since shown that the addition of cobalt to ethanol results in toxicity which is greater than the additive effects of feeding the materials separately (U.S. FDA, 1975b).

In an attempt to provide additional evidence to validate the U.S.S.R. requirement for cobalt in water, Krasovskii and Fridlyand (1971) performed experiments on 380 white mice, albino rats, and guinea pigs, with various oral doses of cobaltous chloride, nitrate, sulfate, and acetate. The LD_{50} of cobaltous chloride was 80 mg/kg of body weight for albino rats and 55 mg/kg for guinea pigs. Aqueous solutions of cobalt at 2.5, 0.5, and 0.05 mg/kg of body weight were administered orally for 6 days/week for 7 months. Cobalt poisoning at 2.5 mg/kg was manifested in disturbed conditioned reflexes and alterations in hematopoiesis. The effects on other metabolic processes and overall resistance were less pronounced. The animals treated with 0.5 mg/kg exhibited only mild and transient polycythemia and a decrease in phagocyic activity of leukocytes. At 0.05 mg/kg, there were no effects on the characteristics investigated.

According to the FDA report (U.S. FDA, 1975), acute cobalt toxicity in some animals has been demonstrated only at very high doses--e.g., in chickens at 50 mg/kg of diet per day and in sheep at 6 mg/kg of body weight per day. At doses under 5 mg/kg of diet or under 2 mg/kg of body weight) no adverse effects were noted. At higher dosages, a loss of appetite, loss of weight, and debilitation were observed. However, intravenous injection of cobalt (at less than 1.0 mg/kg of body weight) causes death. The mechanisms of cobalt's toxic action are not well understood.

Long-term toxicity in humans was observed primarily in children when cobalt was given to correct anemia (U.S. FDA, 1975). Between 1955 and 1961, more than four incidents involving more than 10 children were reported. Children between the ages of 3 months and 12 years became ill as a result of cobalt administration with iron in a commercial preparation. The most common observations were the development of goiter and decreased thyroid function. Increased cardiac rate, increased respiration rate, skin changes, and blood lipid changes were also noted. All symptoms were reversed when cobalt therapy was discontinued. The dosages at which these conditions were observed were between 1-6 mg/kg of body weight per day.

Analysis. With detection limits at 10 µg/liter, cobalt in fresh water is not normally detectable by direct flame atomization; a concentration step is usually required for cobalt determination in water. The U.S. Geological Survey (Brown *et al.*, 1970) uses the APDC solvent extraction procedure, in which the cobalt-APDC complex is extracted at a pH of 2.8 with methylisobutylketone. Similar procedures with APDC have been reported (Brooks *et al.*, 1967; Traversy, 1971; Kinrade and Van Loon, 1974; Aldous *et al.*, 1975). Extractions with diethyldithiocarbamate (Nix and Goodwin, 1970) and dithizone (Sachdev and West, 1969) have also been described. Paus (1971) has used the graphite furnace to enhance atomization of the cobalt in fresh water; the detection limit is 0.4 μg/liter.

Conclusions And Recommendations. Cobalt in natural and treated water has been observed only in trace concentrations--one-hundredth or less of the amounts occurring naturally in foods. It is an essential element for ruminants, in that it allows them to make vitamin B_{12} internally. Apart from its content in vitamin B_{12}, it provides no known nutritional benefit to humans. In doses in excess of 1 mg/kg of body weight it may pose a health hazard to humans, especially children and older people suffering from other ailments. Cobalt acts with alcohol to produce severe cardiac effects at concentrations as low as about 1.2-1.5 mg/liter of beer. The U.S.S.R. has set a limit of 1.0 mg/liter of water (Stoefen, 1973). The Interim Primary Drinking Water Regulations do not list cobalt, nor has the WHO recommended a limit in its International or European standards.

Because the maximum no-observed-adverse-health-effect concentration is more than an order of magnitude greater than that found in any natural water or drinking water supply, there appears to be no reason at present to regulate the concentration of cobalt in drinking water.

The present evidence does not indicate a need to set limits for cobalt in drinking water.

Copper

Occurrence. Copper is frequently found in surface water and some ground water. Copper was detected in 74.4% of over 1,500 river- and lake-water samples in the United States at concentrations up to 280 µg/liter (Kopp and Kroner, 1970). A recent survey detected a maximum of 40 µg/liter in the North Platte River (USGS, 1974). Analysis of 13 Canadian surface and ground waters, including wells, showed copper at 20-860 µg/liter, the maximum in Lake Ontario (Procter & Gamble, 1974). Copper in excess of 100 µg/liter was reported in eight of 27 Illinois streams, with a maximum of 260 µg/liter (Ackerman, 1971). Where higher concentrations of copper are found in raw water, pollution from industrial sources can be suspected.

The effect of treatment and the distribution system pipe material can sometimes produce higher concentrations of copper in finished and tap water than found in the raw-water source. For example, copper tends to increase with the chlorination of water (Shapiro et al., 1962). In Sweden, some water systems actually show a decrease in copper at the tap, compared with raw water (Bostrum and Wester, 1967). This may be a reflection of corrosion control or of the degree to which copper has replaced galvanized iron pipe in household plumbing in the United States since 1950. The survey of the 100 largest cities in the United States showed finished-water copper at less than 0.61-250 µg/liter, with a median of 83 µg/liter. In Denver (Barnett et al., 1969), the metropolitan water system--five water plants with varying raw water sources and treatment processes--showed a relationship between raw- versus finished-water copper concentration, in micrograms per liter, as follows: 25 and 7.6, 67 and 10.6, 4.8 and 4.2, 4.4 and 8.0, and 3.0 and 3.0. The process that almost doubled its copper content consisted of chlorination only. The copper concentration at the consumer tap averaged 0-12 µg/liter. In Washington County, Maryland, 669 copper determinations at the tap were made for both public and private water systems. Efforts were made to sample running water, rather than standing water in contact with plumbing systems overnight, because the latter has been shown to have greatly increased trace-metal concentrations in systems without corrosion control. The correlation coefficients of copper concentration with pH, with hardness, and with conductivity were -0.369, -0.162, and -0.173, respectively; all were significant to p = 0.01 (Oliver, 1974).

The principal anodic and cathodic reactions involving copper in fresh water are known (Camp, 1974). The cold-water corrosion rate of copper tubing as a function of pH is also known; corrosion decreases with increasing pH.

Copper in brass, pipe, and domestic utensils could provide a source of copper in water. Soft, low-pH water could raise the intake of copper by as much as 1,400 µg/day, whereas hard water would reduce the intake (Schroeder *et al.*, 1966).

The copper content of soils varies considerably with the parent rock, weathering, drainage, pH, and organic content. Copper uptake by plants depends on species and is generally quite low in highly organic alkaline soils. The copper concentration in commonly consumed vegetables and leafy plants seldom exceeds 25 ppm and usually is 10-15 ppm. Grains and seeds are good sources of copper, containing about 20-40 ppm. Oysters, clams, crustacea, and the liver and kidneys of animals may contain 200-400 ppm. The human intake of copper in food is estimated to be 2-5 mg/day.

Chemical Characteristics. Copper salts, such as sulfate and chloride, are highly soluble in water with a low pH, but hydrolyze and possibly precipitate copper in water of normal alkalinity. In the normal pH range of natural water containing carbon dioxide, copper might be precipitated as carbonate. This copper salt is soluble at 1.5 ppm in the absence of carbon dioxide. Copper hydroxide, $Cu(OH)_2$, is soluble to the extent of copper at about 1.9 ppm at 25 C. Copper is more soluble than ferric iron and more copper should remain in solution than ferric iron during the weathering and disintegration of rocks under oxidizing conditions. Copper, however, is dissolved and transported less readily than ferrous iron (USGS, 1959).

Metabolism. Copper is recognized as an essential element for both plants and animals. It is a component of several enzymes that perform important physiologic functions. These involve the metabolism of iron and the rate of cell synthesis in the bone marrow.

Copper deficiency has been observed in both man and other animals. It is characterized by anemia, loss of hair pigment, reduced growth, and loss of arterial elasticity. Copper deficiency is not a problem in the United States. The absorption of copper, like all essential elements, from the gastrointestinal tract is limited. Of a daily intake of 2.5 mg, 32% is absorbed. The net absorption is about 5% after fecal and urine excretion. Storage of copper is highest in the liver, kidneys, and intestines.

Health Effects. Copper is a gastrointestinal tract irritant and can be highly toxic. There have been reports (Chuttani *et al.*, 1965) of suicide with gram quantities of $CuSO_4$. Less severe acute episodes have been reported from the ingestion of carbonated beverages that had been in contact with copper tubing or vessels (Hopper, 1958; Semple et al., 1960; Wyllie, 1957; Nicholas, 1968). The doses were 40-50 mg of copper. There is a report of an infant fatality associated with the drinking of water that contained copper at 6.75 mg/liter for 14 months. Whether the child was genetically intolerant to copper is not known (Walker-Smith and Blomfield, 1973). Copper sulfate has been recommended as an emetic at doses (as $CuSO_4$) of 500 mg for adults and 37-50 mg for children (Karlson and Noren, et al., 1965). If vomiting does not occur, these doses are considered to be toxic in children (Holtzman and Haslam, 1968; Decker et al., 1972).

The available evidence does not support chronic toxicity in normal human beings attributed to long-term intake of low (<5 mg) concentrations of copper by mouth (USFDA, 1975). Unlike most animals, sheep are especially sensitive to copper, and a chronic dietary intake of 20-80 ppm is known to be fatal (Todd, 1969; Adamson et al., 1969; Doherty et al., 1969).

The hazard to the general population from dietary copper up to 5 mg appears to be small. A few people are adversely affected by even normal amounts of copper in the diet. This disorder of copper metabolism, Wilson's disease, is inherited as an autosomal recessive trait and leads to hepatic cirrhosis and to necrosis and sclerosis of the corpus striatum (Scheinberg and Sternlieb, 1965). Wilson's disease, formerly fatal within a few years, now can be arrested with chelating agents. There is some concern that any substantial increase in dietary copper intake will result in the conversion of latent cases to overt disease. In addition, there may be a few people who share with sheep the deficiency of glucose-6-phosphate dehydrogenase that is believed to cause hypersensitivity to copper (Salvido et al., 1963).

The only teratogenic effects which may be attributed to copper in mammals are from a deficiency during pregnancy in sheep. It appears that a deficiency or excess of copper has significant consequences for developing embryos (Ferm, 1972).

The interim copper limit of 1 mg/liter of drinking water is based on consideration of taste, rather than toxicity. Depending on individual acuity, the threshold of taste varies from 1-5 mg/liter (Cohen et al., 1974). The European standards for drinking water are also set on the basis of taste and discoloration of fixtures: 0.05 mg/liter at the pumping station and 3 mg/liter after 16 h of contact with plumbing. The international standard sets 0.05 mg/liter as the acceptable limit, with a maximum of 1.5 mg/liter. The USSR also sets 0.05 mg/liter as the acceptable limit. This standard for copper in drinking water is only one-twentieth of the current interim standard. Only 81% of the systems surveyed in the 1975

Interstate Carrier Water Supply System Analysis could have met the 50 μg/liter international standard.

Analysis. Copper content of fresh water can be determined by direct flame atomization, with a detection limit of 2 μg/liter. Copper is extracted with little difficulty by a wide variety of chelate-solvent systems. The U.S. Geological Survey (Brown *et al.*, 1970) uses the APDC-methylisobutylketone extraction of copper from samples at a pH of 2.8. Several other concentration procedures for fresh water have been described (U.S. EPA, 1971; Paus, 1971; Traversy, 1971; Ichinose, 1974; Aldous *et al.*, 1975). Diethyldithiocarbamate can also be used as a chelating agent (Nix and Goodwin, 1970).

The graphite furnace has been used to increase sample atomization by Fernandez and Manning (1971), Paus (1971), Dolinsek and Stupar (1973), Barnard and Fishman (1973), and Surles *et al.* (1975) to implement the determination of copper in fresh water, with a detection limit of 0.05 μg/liter.

Conclusions And Recommendations. At the copper levels found in several extensive water surveys, the potential for toxicity is virtually nonexistent for humans. The current recommended secondary interim standard of 1 mg/liter was only exceeded by 1.6% of 2,595 tap water samples taken in the Community Water Supply Study (EPA, 1970). This value would appear adequate to protect the health of persons from toxicity due to copper in drinking water.

Lead

Occurrence. The natural lead content of lake and river water worldwide has been estimated at 1-10 µg/liter (Livingstone, 1963). Kubota et al. (1974) studied concentrations of zinc, cadmium, lead, copper, and cobalt in rural streams in upstate New York as a model of their distribution under natural geochemical conditions and soil weathering. For lead, average distributions were as follows: soluble lead, 0.12 µg/liter (range, <0.05-0.93 µg/liter); lead in suspended particulate matter, 484 ppm; lead in soil, 7.0 ppm. These and other data suggest that much of the lead in natural water ends up in sedimentary deposits. There is, however, a distinct regional pattern of lead distribution in the United States.

A survey of the mineral content of finished water in the 100 largest cities in the United States was made in 1962 (Shapiro). For lead, the following values were found: maximum, 62 µg/liter; median, 3.7 µg/liter; minimum, not detectable. In another study of raw and finished water in the United States, covering the period 1962-1967, Kopp (1973) reported the following data: frequency of detection, 18.1%; minimum, 1 µg/liter (minimum detectable amount); maximum, 139 µg/liter; mean, 33.9 µg/liter. The corresponding values for raw water were as follows: frequency of detection, 19.32%; minimum, 2 µg/liter; maximum, 140 µg/liter; mean, 23 µg/liter. The increment in the mean value for finished water suggests that lead is picked-up from the plumbing system.

Water samples collected at the tap serviced by 969 water systems throughout the United States indicated an average lead concentration of 13.1 µg/liter (McCabe, 1970). Of the 2,595 samples, 1.4% contained more than the 1962 drinking-water standard of 50 µg/liter, with a maximum of 64 µg/liter.

Important local variations occur, apparently in relation to the use of soft "aggressive" water of slightly acidic pH and the use of lead pipe in service and domestic water lines. Craun and McCabe (1975) have used data from Seattle and Boston to illustrate the effect of "corrosive" water of slightly acidic pH. Both cities use impounded surface water. Chlorination is the only treatment. Comparison between finished-water and tap-water samples showed that in Seattle 95% of the tap-water samples had higher concentrations, with 76% exceeding the limit of 50 µg/liter. In Boston, analyses similarly indicated that lead was being "picked up" in the distribution system; 65% of the tap-water samples exceeded the 50-µg/liter limit for lead. Karalekas et al. (1975) have made further studies in the metropolitan Boston area by collecting multiple water samples from 383 households in Boston, Cambridge, and Somerville, Massachusetts. These cities were selected for study mainly because of the wide use of lead pipe in service lines. Lead concentrations at the tap ranged from <13 to 1,510 μg/liter, with an overall mean of 30 μg/liter. Of the samples collected, 15.4% exceeded the EPA interim drinking-water standard of 50 μg/liter. In all cases, the lead content of drinking water was higher at the tap than at the treatment plant. Highest concentrations were found in early-morning samples, with the lowest mean concentrations in running water and intermediate values in standing water and in composite samples obtained throughout the day. The mean concentration for composite samples was 53 μg/liter; for this type of sample, 26.7% exceeded the standard of 50 μg/liter. The percentage of households exceeding the standard was greater in the Boston (25.5%) and Somerville (30%) areas than in Cambridge (14.5%). This was attributed to some differences in the overall composition of the different water supply systems.

Available data generally indicate that the addition of lead to drinking water occurs chiefly in the distribution system, including household plumbing, and that this is most likely to occur in areas with soft "aggressive" water.

Craun and McCabe (1975) have reported that the average intake of lead from drinking water by adults may be estimated at 26 μg/day, using the following assumptions: the average lead concentration in tap water is 13 μg/liter and the daily water consumption by adults is 2 liters. In children, water intake is related to caloric requirements, water absorbed from food, and the need to maintain a dilute urine. On the basis of body weight, water requirements are 2-3 times higher in children than in adults. If one assumes a heavy use of dehydrated powdered food and beverages for infant feeding, with reconstitution entirely with tap water, then lead intake from tap water in a 6-month-old infant may be as high as half the adult intake as estimated above, or 13 μg/day. Generally, for children under 3 years of age who receive either a mixed diet or concentrated liquid formula, the amount of tap water used may be estimated at up to 500 ml/day, which would provide a lead intake of up to 6 μg/day, if one uses the average figure of 13 μg/liter of drinking water at the tap. For young children, this is small, in comparison with food lead.

For the general adult population, the lead content of foods is the major source of exposure. Kehoe (1961) estimated from fecal lead data in studies carried out primarily in men that daily dietary lead intake is approximately 300 μg/day. More recently, Tepper and Levin (1975) concluded that in women, 100 μg/day is a closer approximation of current dietary intake. For the adult, balance studies have indicated that absorption of dietary lead is approximately 5-10%.

Kolbye et al. (1974) used "market basket" data and other information available to the FDA and estimated for 2-y old children that dietary intake is approximately 100 µg/day. Limited balance data (Alexander et al., 1973) indicated an average intake of 9-10 µg/kg or 100-150 µg/day for 2-3-y old children. More important, these balance data in children are consistent with those in young growing animals and indicate that approximately 40-50% of dietary lead is absorbed and that 20-25% is retained. Although the distribution of retained lead between bone and soft tissue cannot be determined from these balance studies, autopsy data (Barry, 1975) show a steady increase in bone lead throughout the first 15-20 years of life. For children, ingestion of soil and exposure to household dust in old houses are important additional sources of lead intake (Ter Haar and Aronow, 1975; Sayre et al., 1974).

Residents near stationary point sources also constitute special lead-exposure groups (Landrigan et al., 1975).

In summary, it appears that, at an average drinking-water lead concentration of 13 µg/liter, lead intake from drinking water constitutes about one-tenth or less of that obtained from an ordinary diet.

Chemical Characteristics. Lead occurs in rocks primarily as the sulfide (galena) and in the form of oxides. It may replace some ions, such as calcium. Lead also occurs in potassium feldspar, where it replaces potassium. Lead carbonate is common in the oxidized zone of lead ores.

Lead sulfate is reported to be soluble in water to the extent of 31 ppm at 25°C. In natural water, this concentration is not approached, however, because a pH of less than 4.5 would probably be required to prevent the formation of lead hydroxide and carbonate. In natural water containing bicarbonate and carbonate alkalinity, the concentration of lead is usually limited by the solubility of lead carbonate. It has been reported that at 13°C water free of carbon dioxide will dissolve the equivalent of lead at 1.4 ppm; the solubility is increased nearly fourfold by the presence of carbon dioxide at 2.8 ppm. The presence of other ions may increase the solubility of lead. It is likely that lead is adsorbed by minerals in sediments and soils, so that the observed concentrations rarely reach the theoretical limit (USGS, 1959).

Lead may be dissolved from water pipes most readily by water that is low in hardness, bicarbonate, and pH and high in dissolved oxygen and nitrate.

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The chemical forms and physical states in which lead and other trace metals occur in raw water are not well known. Recent reports (Guy and Chakrabarti, 1975a; Ramamoorthy and Kushner, 1975) suggested that the problem is complex and may well vary from one body of water to another. It has been demonstrated that some aquatic organisms can convert inorganic mercury and arsenic to alkyl compounds. Recent preliminary data (Wong *et al.*, 1975) suggested that under rather unnatural experimental conditions it is possible to alkylate lead. Wood (1976) has summarized basic conditions for the alkylation and reductive dealkylation of heavy metals in aquatic systems. He noted that the vitamin B_{12} found in microorganisms holds a unique position in aqueous systems for the alkylation of heavy metals. Conversely, cytochromes may be important in the reduction of metal ions to elemental form. These considerations are not cause for alarm, but they do indicate the need for research in this field. Progress may well depend on improved analytic techniques, if the total metal content is to be fractionated into its various constituents.

Metabolism. The absorption of dietary lead is 5-10% in the adult (Kehoe, 1961) and 40-50% in children 2-3-y old (Alexander *et al.*, 1973). No data are available for very young infants, but animal data indicate that the percentage absorbed is age-related and may be higher in early infancy (NAS, 1976). Absorbed lead is excreted through both the kidneys and the intestinal tract. Long-term balance studies by Kehoe (1961) in adults suggested that adults are in balance. However, these data derived from a few people must be weighed against autopsy data, which indicate that, although soft-tissue lead concentrations remain stable in adults, bone lead content may increase with age, at least to the age of 40 or 50 years (Barry, 1975; Gross *et al.*, 1975). Bone is the storage site for at least 90% of the total lead body burden in adults and approximately 70% in growing children.

Health Effects. No beneficial effects of lead have yet been found. Acute lead poisoning is extremely rare, if it occurs at all in the general population. One child was estimated to have consumed approximately 1 g of lead per day in fruit juice during the 5 weeks immediately before his death (Klein *et al.*, 1970). In one adult, estimated to have consumed approximately 2 mg of lead per day, at least a year and a half elapsed before the onset of acute symptoms of plumbism (Harris and Elsea, 1967). Lead apparently does not cause gastrointestinal symptoms within a few hours, as is the case of acute poisoning due to ingestion of cadmium, iron, or other heavy metals and metalloids (such as arsenic).

The induction of renal tumors with lead has been demonstrated in rats but not in other species. Similarly, mutagenic and teratogenic effects have been reported in experimental systems

(NAS, 1972). However, none of these effects have been documented in man. The main chronic adverse effects of lead are those produced in the hematopoietic system, central and peripheral nervous system, and kidneys. Although disturbance in heme synthesis is considered to be the critical or first adverse effect of lead (Nordberg, 1976), measures of comparable sensitivity for the detection of disturbances in nervous system metabolism are not available.

Zielhuis (1975a,b) has summarized available data on dose-response relationships for lead in man. Currently, the most sensitive effect is that on heme synthesis. There is a detectable and statistically significant increase in red-cell protoporphyrin in women and children, as blood lead concentration increases above about 25-30 µg/dl (Roels, 1975; Zielhuis, 1975 a,b). In men, increase in red-cell protoporphyrin apparently does not occur until blood lead concentration exceeds about 35-40 µg/dl of whole blood. Zielhuis (1975a,b) and Albert et al. (1974) reviewed clinical data and suggested that the no-effect concentration of lead in the developing human nervous system is approximately 55-60 µg/dl of whole blood as judged by clinical outcome. However, animal data have suggested that this value may be lower (Brown, 1975; Carson et al., 1974). In addition, Lancranjan et al. (1975) reported evidence of disturbance in reproductive function in occupationally exposed men with blood lead concentrations in excess of about 50-80 µg/dl of whole blood. These disturbances included alterations in spermatogenesis (asthenospermia, hypospermia, teratospermia) through a direct toxic effect of lead on the gonads. Comparable data for women are not available.

Preliminary clinical data from soft-water areas in Boston and in Scotland are now available and suggest a relationship between lead in tap water and blood lead concentration. The consumption of soft water from the acidic moorlands of Scotland and northern England has been associated with clinical cases of lead poisoning (Bacon et al., 1967; Beattie et al., 1972). In these cases, it appears that soft well water or rainwater was not only conveyed in lead pipes, but also stored in lead-lined cisterns. In these clinical cases, the lead concentration in tap water ranged between 570 and 3,136 µg/liter. Such water had apparently been consumed over a number of years; those involved were adults. This led Moore et al. (1975) to make a more intensive study in Glasgow, Scotland. In a study of 23 Glasgow households, significant associations were found between water lead content, length of lead piping, and use of a lead-lined storage tank.

Studies in Edinburgh, although showing a relationship between blood lead and tap-water lead, did not fully confirm the data from Glasgow. In Edinburgh, households with copper plumbing were compared with households with lead plumbing. The early-morning sample of water was drawn to clear the lines and then discarded, and a sample drawn later in the day was used. Water lead concentration in homes with copper plumbing was less than that in homes with lead plumbing, and blood lead content could be correlated with water lead content.

In the Boston area, Craun and McCabe (1975) reported preliminary data indicating that, when household water lead content in the sample obtained during the day exceeded 100 μg/liter and the data were controlled for proximity to traffic density, a significantly increased frequency of blood lead concentrations in excess of 35 μg/dl was found. These human studies--although they involved a small number of subjects and were not controlled for sex, age, and smoking habits--suggested that drinking water with a lead content greater than 100 μg/liter may be sufficient to raise and sustain blood lead concentrations at above 25 μg/dl whole blood. This is the blood level which has been shown to be the apparent threshold for the increased red-cell protoporphyrin.

In a provocative retrospective study, Beattie *et al.* (1975) investigated 77 2-5-y old mentally retarded children and 77 nonretarded healthy control children, matched for age, sex, and geographic location in the city of Glasgow, Scotland. They did not have blood lead data obtained at an earlier, more vulnerable period of development. They concluded that a child exposed during gestation and early infancy to a water lead content greater than 800 μg/liter "is at least 1.7 times (and probably a much greater factor) more likely to be mentally defective than a child whose exposure to water-lead is completely unknown."

Experimental data strongly indicate that among human populations the fetus and young child, particularly under 3 years old, are at increased risk of adverse effects due to lead. This is based on both a higher rate of intestinal absorption and a high rate of brain growth and maturation. Animal data further suggest that absorption and other dietary components play a very prominent role in this susceptibility (NAS, 1976). In addition, people with chronic renal insufficiency and metabolic disturbance in bone homeostasis and possibly those with zinc deficiency may be at increased risk. There are, however, no data on humans to substantiate these latter hypotheses.

Analysis. The analysis of lead in biologic samples is fraught with difficulties. The great variations in comparisons between and within laboratories are well known (Lauwerys *et al.*, 1975).

The detection limit with direct flame atomization is 10 µg/liter. As for most trace metals in water, solvent extraction is the method of choice for concentration. The U.S. Geological Survey (Brown *et al.*, 1970) uses APDC-methylisobutylketone extraction at a pH of 2.8, and this procedure has been used by other investigators (Brooks *et al.*, 1967; U.S. EPA, 1971; Paus, 1971; Traversy, 1971; APHA, 1971; Everson and Parker, 1974; Kinrade and Van Loon, 1974). Diethyldithiocarbamate has also been used to extract lead (Nix and Goodwin, 1970).

Specialized aspiration procedures may be used to improve lead detection limits. The sampling boat and Delves cup have a limit of 1 µg/liter (they use 1- and 0.1-ml samples, respectively) and have been applied to water analyses by Kerber and Fernandez (1971), Paus (1971), and Maine *et al.* (1975).

The graphite furnace will increase sample atomization and can be used to increase detection to as little as 0.05 µg/liter and has been used for fresh-water analyses by Paus (1971), Fernandez and Manning (1971), Dolinsek and Stupar (1973), Barnard and Fishman (1973), Rattonetti (1974), and Surles *et al.* (1975).

Conclusions And Recommendations. If one uses the critical toxic effect approach to preventive medicine (Nordberg, 1976), then a water lead content of 100 µg/liter at the household tap is probably not acceptable. "The critical toxic effect is defined as the most sensitive and specific biological change which is outside of acceptable physiological variation" (Nordberg, 1976). Preliminary data suggest that the present limit of 50 µg/liter may not, in view of other sources of environmental exposure, provide a sufficient margin of safety, particularly for fetuses and young growing children. Although further studies will be necessary to arrive at a reasonable limit, it is suggested that the limit be lowered. This recommendation is made with the assumption that analytical methodology will be sufficient to detect this value above background.

1. A further elucidation of the neurochemical disturbance caused by lead is a basic research need, which should be worked out in appropriate animal models and followed with confirmatory clinical and epidemiologic studies, where possible. Much experimental evidence points to significant interactions between lead, copper, zinc, iron, calcium, and magnesium. These interactions are high-priority items, although their significance may pertain more to nutrition and genetic susceptibility than to drinking water itself.
2. Definitive studies in soft-water areas in relation to the influence of lead contents in the distribution system and measures for its control deserve the highest priority, insofar as drinking-water quality is concerned.

3. The question of whether or not lead can be alkylated by aquatic organisms in relation to drinking water deserves high priority.

4. There are no data on illness of human infants between birth and 1 year of age as related to lead. Dose-response data for this group, as well as for pregnant women, are urgently needed, to provide a base for estimating overall safe levels of lead exposure for these highly susceptible population groups. Dose-response data are also needed throughout the preschool years; there are very few data that satisfy both epidemiologic and toxicologic criteria for dose-response data in this group.

5. The needed data depend heavily on on the availability of precise and accurate analytical measurements. Substantial improvements in methods are needed. Electrochemical approaches appear to be the most promising in this regard, including anodic stripping voltammetry and differential pulse polarography.

Magnesium

Occurrence. In view of the geologic abundance, high solubility, and numerous industrial uses of magnesium, it is not surprising that seawater contains about 1,350 mg/liter. The average for natural fresh water is about 4 mg/liter. In a survey of finished water in public supplies of the 100 largest cities in the United States, Durfor and Becker (1964) reported a median concentration of 6.25 mg/liter, a maximum of 120 mg/liter, and a minimum of nil.

The U.S.P.H.S. drinking-water standards of 1925 included a maximum recommended magnesium concentration of 100 mg/liter. This limit was raised to 125 mg/liter in the 1942 and 1946 standards, but it was deleted in the 1962 standards. According to Stoefen (1973), the U.S.S.R. has not set a limit on magnesium; however, the World Health Organization has established European and International desirable limits ranging from 30-125 mg/liter, depending on the sulfate concentration. If the sulfate exceeds 250 mg/liter, the magnesium is limited to 30 mg/liter. The WHO specifies an absolute maximum of 150 mg/liter for magnesium in drinking water.

Several categories of foods are rich in magnesium—e.g., nuts, about 1,900 mg/kg; cereals, about 800 mg/kg; seafoods, about 350 mg/kg; meat, about 260 mg/kg; legumes, about 240 mg/kg; vegetables, about 170 mg/kg; and dairy products, about 150 mg/kg. Fruits, refined sugars, and fats are low in magnesium (Schroeder *et al.*, 1969).

Chemical Characteristics. Magnesium is one of the most common elements in ores, minerals, rocks, and soil. It constitutes about 2.1% of the earth's crust and ranks eighth among the elements in order of abundance. Because it is very active chemically, it is not found in the elemental state in nature. Most of its salts are very soluble; even the carbonate will dissolve to the extent of 100-300 mg/liter at 20°C. On the basis of the solubility product of magnesium hydroxide at 18°C, magnesium ions theoretically can be present in the following amounts: 28,000 g/liter at pH 7, 28.8 g/liter at a pH of 10, and 0.288 g/liter at a pH of 11. This solubility phenomenon is useful in treatment processes to remove magnesium from water; but, insofar as natural waters are concerned, it is described here merely to show that at common pH values magnesium ions may be present at concentrations of many grams per liter in dissolved form (McKee and Wolf, 1967).

Metabolism. Magnesium is an essential element in human and animal nutrition and also in plants, for it is a component of all types of chlorophyll. It is the most abundant intracellular divalent cation in both plants and animals. It is an activator of many mammalian enzymes. Magnesium deficiency in humans and animals depends on many factors. It occurs in alcoholics, persons performing hard labor in hot climates, those with some endocrine disturbances, and patients using potent diuretics. Excessive magnesium in the body (hypermagnesemia) occurs in humans primarily as a result of severe kidney disease.

The average adult American ingests between 240 and 480 mg of magnesium per day in food and water. Magnesium intakes of 3.6-4.2 mg/kg of body weight per day are thought to be adequate to maintain magnesium balance in normal adults (Jones et al., 1967). The recommended dietary allowances for magnesium are 300 mg/day for women, 350 mg/day for men, and 150 mg/day for children (Coussons, 1969). The nutritional value of magnesium supplements beyond these levels has not been established.

According to Szostak (1961), magnesium is one of the most important electrolytes in the body. In adults, the body content averages about 25 g (or about 350 mg/kg of body weight) and can vary from 21-28 g.

The tissues contain 98% of the body content of magnesium, with the other 2% found in extracellular fluids. The concentration of magnesium in plasma averages 21.6-25.2 mg/liter, with a normal range of 16.8-30 mg/liter. The greatest amount of magnesium is found in the skeleton, which contains more than half the magnesium stored in the body. For normal people on regular diets, the average daily absorption of available magnesium from the gastrointestinal tract is about 30-40%.

Aikawa et al. (1958) administered magnesium-28 orally to 26 human subjects. They found that fecal excretion within 120 h accounted for 59-88% of the administered dose. Less than 10% of the radioactivity was recovered in the urine within 72 h. The low renal excretion was thought to be due to poor gastrointestinal absorption.

Normally, the kidney is the major excretory pathway for magnesium, once it is absorbed. Hence, the kidney is the organ primarily responsible for regulating the total human body content of magnesium.

According to Consolazio et al. (1963), when men were exposed to desert temperatures for several days, 10-15% of the total output of magnesium occurred in perspiration. Under extreme conditions, sweat can account for 25% of the daily magnesium excretion; this could lead to hypomagnesemia.

Health Effects. Magnesium salts at levels over 700 mg/liter (especially magnesium sulfate) have a laxative effect, particularly on new users, although the human body can adapt to the effects of magnesium with time (McKee and Wolf, 1963). The most sensitive people are affected by $MgSO_4$ at about 400 mg/liter, and the average person, at about 1,000 mg/liter (Kehoe, 1953). Magnesium salts (principally magnesium hydroxide) are used extensively as antacids and laxatives. The usual therapeutic doses are 5-15 ml of a 7-8.5% solution of magnesium hydroxide and at least 250 mg of magnesium oxide (Goodman and Gilman, 1975).

Magnesium in water is not considered a public-health hazard, because the taste becomes quite unpleasant before toxic concentrations are reached (Negus, 1938). The taste threshold for magnesium has been reported by Lockhard _et al._ (1955) as 100 mg/liter in sensitive persons and about 500 mg/liter for the average person (Kehoe, 1953).

A thorough discussion of the role of magnesium in the human body is presented by Szostak (1961) but such detail is beyond the scope of this report. It is sufficient to note here that magnesium is an essential element in human nutrition, that most diets contain adequate amounts of magnesium, that hypomagnesemia occurs frequently in ruminant animals and occasionally in humans under stress, and that hypermagnesemia occurs in humans only as a result of kidney malfunction.

Analysis. According to Standard Methods (APHA.,1971), three methods are used for the determination of magnesium in water. A gravimetric method can be used, but only after the removal of calcium salts.

Magnesism in water can also be determined by atomic absorption spectrophotometry, with a sensitivity of 15 μg/liter, and by photometry with a sensitivity of 100 μg/liter.

Conclusions And Recommendations. Magnesium is an essential element in human, animal, and plant nutrition. Excess magnesium in the diet is seldom harmful, for it is generally excreted in the feces. High concentrations of magnesium sulfate in drinking water may have a cathartic effect on new users, but persons usually adapt to these levels with time. Excessive magnesium in body tissues and extracellular fluids occurs only as a result of severe kidney malfunction. Magnesium deficiency in humans may occur in alcoholics, persons performing hard labor in hot climates (because magnesium is excreted in sweat), those with some endrocrine disturbances, and patients using potent diuretics. Such deficiencies can best be overcome by oral administration of magnesium compounds.

The National Interim Primary Drinking Water Regulations contain no limit for magnesium, nor did the 1962 USPHS Drinking Water Standards. The U.S.S.R. has set no limit, but the WHO has recommended a maximum of 150 mg/liter. In view of the fact that concentrations of magnesium in drinking water less than those that impart astringent taste pose no health problem and are more likely to be beneficial, no limitation for reasons for health appears needed.

Manganese

The NAS-NRC (1973) has reviewed the medical and biological effects of manganese; that work has been reviewed and evaluated for this report, and some sections are quoted here. The EPA has also discussed (1975) manganese, and portions of its review are cited.

Occurrence. Durum and Haffty (1961) observed a maximum manganese concentration of 181-185 μg/liter in two different surface waters. The median for all samples was 20 μg/liter. Kopp and Kroner (1967) detected manganese in 51.4% of surface-water samples; the concentration ranged from 0.3-3230 μg/liter, with a mean of 59 μg/liter. A maximum of 1,200 μg/liter was detected in two different surface waters in 1974 (USGS., 1974).

Chemical Characteristics. In chemical behavior and occurrence in natural water, manganese resembles iron. However, manganese is much less abundant in rocks. As a result, the concentration of manganese in water generally is less than that of iron.

Manganese occurs in more than one oxidation state. The oxidation states of manganese to be expected in water are Mn^{+2} and Mn^{+4}. Manganese can also occur in more highly oxidized states (such as permanganate, MnO^-), but is not normally encountered in those forms in natural water. Under reducing conditions, manganese goes into solution in water containing carbon dioxide, as manganous ion.

Manganous ion is more stable in water in the presence of oxygen than is ferrous ion under similar conditions. The presence of organic matter in water stabilizes manganous solutions, perhaps owing to formation of complex ions by organic compounds.

"Total" and "dissolved" manganese are reported separately in most water analyses. The difference between the two is likely to be less significant for manganese than for iron, but the same problem exists in determining actual conditions in the aquifer on the basis of "dissolved" manganese values. The "total" manganese values are better for determining these conditions, even though the manganese may be partly in the form of colloidal oxide and hydroxide by the time it is determined.

Manganese concentrations greater than 1 mg/liter may result where manganese-bearing minerals are attacked by water under reducing conditions or where some types of bacteria are active.

Metabolism. The divalent manganese ion activates many enzyme reactions involved in carbohydrate breakdown and in the metabolism of organic acids, nitrogen, and phosphorus.

Manganese metabolism is regulated by the adrenal glands. Ingested manganese is absorbed through the intestine and is concentrated in the liver. Although manganese may be distributed to the tissues, most of the excess is discharged via the bile or by other gastrointestinal routes, thereby keeping the manganese concentration in various tissues relatively stable (U.S. EPA, 1975). A small percentage of the manganese excreted into the intestines is reabsorbed and transported in the plasma in its trivalent form. Fast and slow components of the manganese disappearance curves have been identified that have respective half times of 4 days and 39 days in humans (U.S. EPA, 1975). Inorganic manganese excretion is almost exclusively fecal. However, the organic form is excreted in both feces and urine (U.S. EPA, 1975).

Manganese is found in minute concentrations in the cells of all living things and has been established as essential to a wide variety of organisms, including bacteria, plants, and mammals.

Manganese is widely distributed throughout the body; concentrations are characteristic for the various organs and vary little within or among species.

> Higher concentrations of manganese are generally associated with pigmented portions of the body including retina, pigmented conjunctiva, dark hair, and dark skin. The pituitary gland, pancreas, liver, kidney, and bones normally have higher concentrations of manganese, and skeletal muscle has a very low concentration.... The storage capacity of the liver for manganese is limited and offers a contrast in this regard with iron and copper.... Human livers from healthy people of all ages contain manganese at about 6-8 ppm (dry-weight basis). In contrast with many other trace metals, manganese does not accumulate significantly in the lungs with age, averaging about 0.22 ppm in aged man (p. 80-81, NAS, 1973).

In foods consumed by humans, the highest concentrations of manganese are found in nuts, tea, and spices (U.S. EPA, 1975). The average daily consumption of manganese for man is from 3-7 mg (NAS, 1973). Of the trace metals, manganese is third in the proportion of intake from water as compared to food (Craun and McCabe, 1975).

Health Effects. Manganese is an essential trace nutrient for microorganisms, plants, and animals, including all species of

> mammals and birds that have been investigated. Manganese deficiency has been observed in many mammalian species, both under field conditions and in the laboratory. It is therefore reasonable to conclude that man also has a nutritional requirement for manganese. The incidence of human manganese deficiency has not been investigated, nor has it been determined whether such a deficiency is a health hazard to man. Moreover, minimal human nutritional requirements have not been established. It will be necessary to determine such requirements if desirable limits of exposure to dietary and environmental manganese are to be established (NAS, 1973, pp.97).

Manganese is a coenzyme in many mitochondrial reactions. Examples of non-specific manganese-activated enzymes include hydrolases, kinases, decarboxylases, and transferases. Some enzymes such as succinic dehydrogenase have an absolute requirement for manganese (NAS, 1973).

"Acute manganese poisoning is extremely rare. Chronic exposure is seldom fatal but may result in permanent crippling. Diagnosis is difficult unless a history of exposure of at least three months is present. The symptoms are sleepiness, muscular twitching, leg cramps, increased tendon reflexes, a peculiar characteristic spastic gait, emotional disturbances, and a fixed mask-like expression" (U.S. EPA, 1975, p. 6-1). "The toxicity of specific manganese compounds appears to depend upon the type of manganese ion present and the oxidation state of the manganese; the divalent manganese cation is reported to be 2-1/2-3 times more toxic than the trivalent cation" (U.S. EPA, 1975, p. 6-2)

Manganese has a very low order of acute oral toxicity. When rats are given 2000 ppm in their diets growth is unaffected, and hens can tolerate 1000 ppm without ill effects, but 4800 ppm is toxic to young chickens (NAS, 1973).

Chronic manganese poisoning almost always is the result of inhaling high concentrations of manganese dust. The symptoms appear after several months and are often reversible if exposure is terminated. Even with an inhalation exposure there is some evidene that a large amount of manganese enters the body through intestinal absorption (EPA, 1975). Chronic manganese poisoning is characterized by progressive deterioration of the central nervous system; the effects are not completely reversible.

> There is currently no evidence that human exposure to manganese at the levels commonly observed in the ambient atmosphere results in adverse health effects. The only human health effects attributable to manganese in ambient air were found in persons living in the immediate vicinity of two

> major point sources in Norway and Italy. Manganese pollution is presently a local problem, but the widespread use of manganese fuel additives would make man-made emissions more ubiquitous. There is no evidence that predicted manganese concentrations resulting from the use of methylcyclopentadienyl manganese tricarbonyl would result in adverse health effects; however, respiratory irritant effects from long-term or frequent exposure to low concentrations have not been thoroughly investigated. Most effects from manganese in humans appear to result from prolonged inhalation. Manganese pollution of water does not appear to be a problem except possibly in isolated cases of waste disposal. Atmospheric concentrations of manganese observed in urban areas can be attributed primarily to man-made sources. The principal source of atmospheric emissions is metallurgical processing (U.S. EPA, 1975, p. 2-3).

> Chronic exposures to high levels of manganese increase hemoglobin values and erythrocyte counts, which indicates that manganese stimulates production of erythrocytes, as does iron-deficiency anemia. Recovery from anemia caused by improper nutrition is much prompter following the administration of ferrous sulfate and manganese chloride than of ferrous sulfate alone, which demonstrates the relationship between the effect of manganese on erythrocyte production and the intestinal absorption of manganese in anemic individuals (U.S. EPA, 1975, p. 6-2).

The neurologic manifestations of manganese poisoning appear to be caused mainly by inhalation of dust or fumes, with ingestion as an additional factor. An acute waterborne epidemic was reported in Japan in 1941.

> An encephalitis-like disease occurred in six members of a family. All had the same symptoms, including loss of appetite, constipation, and a mask-like facial expression, with running saliva. Tonicity of muscles was decreased; the leg joints were painful and rigid; the arm muscles showed rigidity and tremors; there was temporary double vision; tendon reflexes were increased; and there was some mental disturbances, memory loss, and melancholia.... One victim had died, two were hospitalized, and three were up and about. Blood and spinal fluid samples were sterile, with normal cellular counts. Histologic examination of the autopsy material from brain and spinal cord showed no signs of encephalitis....

> Symptoms pointed strongly to some form of intoxication.... It war learned that the family maintained a bicycle-repair shop and that many old dry cells for bicycle lamps had been

> buried near a well that supplied water for the family. It was presumed that the intoxication was caused by manganese, which, with zinc, is a principal constitutent of the cells. [Well-water] analysis showed unusually high concentrations of manganese and zinc in the water from this and several other wells. Manganese and zinc were found in large quantities in the viscera of the autopsied victims and in the blood and urine of survivors. Ten more patients were discovered among the neighbors of the family; all had drunk the contaminated water. (NAS, 1974, pp. 109-110).

Both acute and chronic effects of manganese poisoning, are similar to Parkinson's disease. There appears to be some similarities between the clinical features of the extrapyramidal disease of manganism and those of Parkinsonism, There is some indirect evidence that chronic manganism and Parkinsonism may have similar biochemical abnormalities with respect to the extrapyramidal system.

It has been shown that levels of dopamine, one of the chemicals that functions in transmission of nervous impulses, are reduced in discrete areas of the brain in Parkinson's disease. L-Dopa, the precursor of dopamine, can cross the blood-brain barrier and be converted to dopamine in the brain. L-Dopa has proved to be quite beneficial in Parkinson's disease. It has has also been successfully used as therapy in persons with chronic manganism, which has been associated with decreased brain dopamine (NAS, 1974).

Analysis. The manganese detection limit by direct flame atomization is 2 µg/liter. However, solvent extraction is used for many determinations. Analytic conditions are more critical for the extraction of manganese than for most other metals, because many manganese-chelate complexes are unstable in solution. With pH control and immediate analysis after extraction, accurate determinations are possible. The U.S. Geological Survey procedure (Brown *et al.*, 1970; and Aldous *et al.*, 1975) uses the extraction of the manganese-APDC complex with methylisobutylketone at a pH of 6.0 with immediate aspiration of the extract. Yanagisawa *et al.* (1969) and Jenne and Ball (1972) have studied the stability of manganese chelates. When the graphite furnace is used to increase sample atomization, the detection limit is lowered to 0.01 µg/liter. Fernandez and Manning (1971), Barnard and Fishman (1973), Surles *et al.* (1975), and Shigematsu *et al.* (1975) have described its application to fresh-water analysis.

Conclusions And Recommendations. Manganese is an essential trace element for man. It plays an important role in many enzyme systems. Manganese toxicity has been associated with airborne exposure, but chronic toxicity from drinking water has not been reported. With surface water averaging less than 0.05 mg/liter in several surveys, the potential for harm from this source is virtually nonexistent.

The main problem with manganese in drinking water has to do with undesirable taste and discoloration of the water. The WHO (1970) suggests that such problems may arise at concentrations of manganese greater than 0.05 mg/liter, the same limit recommended by the U.S.P.H.S. (1962).

The manganese report from NAS (1973) suggested several research priorities for gaining a better understanding of manganese toxicity. Some of the questions which need answers include the following:

> 1. Is there individual human susceptibility to excessive or deficient concentrations of manganese? If so, how can it be detected, and how can it be predicted? Are the differences due to diet, genetic makeup, concomitant stress, variations in absorpton, disease, or interactions with drugs and chemicals? Are there also group differences?
> 2. What are the effects on pregnant women and infants of chronic excessive exposure to manganese? Is the fetus at risk?
> 3. What controls the metabolism and turnover of manganese?
> 4. What accounts for the time course of the symptoms in manganism? Why do the psychiatric symptoms precede the neurologic?
> 5. With few exceptions, manganese pollution does not occur in isolation from pollution from other substances. How do these pollutants interact? Are their effects merely additive, or do some combinations create special hazards to health?
> 6. Are the so-called lower oxidative states more toxic than the higher ones? This has often been reported but has not been proved. Indeed dose-response relations have not been established for any manganese compound. Does the toxicity of manganese depend on its physical form?
> 7. Further research is needed to determine the clinical value of present tests of blood, urine, and hair as indices of recent absorption of excessive manganese. Does increased manganese content of any of these samples correlate with later features of manganese toxicity? (NAS, 1973, pp. 137-138).

Mercury

Occurrence. Mercury is one of the least abundant elements in the earth's crust, being seventy-fourth in a list of 90. Greater than trace amounts are found in at least 30 ores, but in only one, the sulfide cinnabar, does the concentration justify commercial extraction.

A major use of mercury has been as a cathode in the electrolytic preparation of chlorine and caustic soda; this accounted for 33% of total demand in the United States in 1968. Electrical apparatus (lamps, arc rectifiers, and mercury battery cells) accounted for 27%, and industrial and control instruments (switches, thermometers, and barometers) and general laboratory applications accounted for 14% of demand. Use of mercury in antifouling and mildewproofing paints (12%) and mercury formulations used to control fungal diseases of seeds, bulbs, plants, and vegetation (5%) were other major sources of demand. The remainder (9%) was for dental amalgams, catalysts, pulp and paper manufacture, pharmaceuticals, and metallurgy and mining (Wallace *et al*, 1971). Because of associated environmental hazards, the U.S. EPA in February 1976 canceled registrations for all pesticide products containing mercury used as bactericides and fungicides in paints and coatings, on turf, for seed treatment, and for any other use not specifically permitted (U.S. EPA, 1976).

According to data published by the U. S. Geological Survey in 1970 (U.S.G.S., 1970) mercury concentrations in broad categories of rocks ranged from 0.01-20 ppm. Igneous rocks generally contain less than 0.2 ppm and sedimentary rocks generally average less than 0.10 ppm, except for organic rich shales, which may have concentrations of 10 ppm or more.

Seawater contains 0.03-2.0 µg/liter, depending on the sampled area, the depth, and the analyst. In a study of Pacific waters, mercury concentrations were found to increase from surface values of near 0.10 µg/liter to 0.15-0.27 µg/liter at greater depths. In an area seriously affected by pollution (Minamata Bay, Japan), values ranged from 1.6-3.6 µg/liter. Oceanic mercury is generally present as an anionic complex (HgCl$^{=}$), which does not have as pronounced a tendency to bind to particulate substances and then settle out as do mercury compounds found in fresh water (Wallace *et al*., 1971).

Little attention was paid to mercury in water in the United States before 1970. A 5-year summary (1962-1967) of trace metals in rivers and lakes of the United States prepared by the U.S. Department of the Interior, Federal Water Pollution Control Administration did not include mercury among the trace elements reported (Kopp *et al.*, 1967). The Department of the Interior carried out a nationwide reconnaissance of mercury in U.S. water in the summer and fall of 1970 (Jenne *et al.*, 1972). Of the samples from the industrial wastewater category, 10% contained mercury at greater than 10 μg/liter; nearly 0.5% of the samples in this group contained more than 1,000 μg/liter. Only 4% of the surface-water samples contained in excess of 10 μg/liter. The higher mercury concentrations were generally found in small streams. About half the 43 samples from the Mississippi River contained less than 0.1 μg/liter. The mercury content of lakes and reservoirs was between 0.1 and 1.8 μg/liter. With few exceptions, the mercury content of groundwater samples was below detection (0.1 μg/liter).

In a survey done by the EPA Division of Water Hygiene, 273 community, recreational and federal installation water supplies were examined. Of these 261 or 95.5% showed either no detectable mercury or less than 1.0 μg/liter in the raw and finished water. Eleven of the supplies had mercury concentrations of 1.0 - 4.8 μg/liter and one supply exceeded 5.0 μg/liter. When this one supply was extensively reexamined the mercury concentration was found to be less than 0.8 μg/liter (Hammerstrom *et al.*, 1972).

The combined effects of treatment and distribution on trace elements, including mercury, were investigated in the municipal water systems of three cities in Sweden. The concentration range for mercury was 0.09-5.4 μg/liter in raw water, and it remained unchanged in tap water in the three systems. (Andelman, 1974)

All vegetable materials naturally contain traces of mercury, the actual amount depending on the locality from which the sample was taken, the species, and other factors. The mercury concentrations in plant materials generally range from 0.10 ppm down to 0.01 ppm or even less; but higher concentrations are found and may be caused by naturally high concentrations of mercury in the soil.

Background concentrations of mercury in animals are difficult to assess, particularly for terrestrial samples, because former agricultural uses of mercury products were so widespread and uncontaminated sources so rare. Data from the literature suggest that normal values for eggs and the flesh of birds and animals are generally less than 0.02 ppm. Marine fish have mercury concentrations usually below 0.10 ppm and nearly always below 0.15 ppm, but this depends very much on species as swordfish may contain more than 1 ppm. Concentrations of 0.20 ppm or less are assumed normal for fresh-water fish, but once again this depends on species and region. The higher background concentrations in fish as compared to other animals, fruits, and vegetables are due to the marked ability of fish to accumulate methylmercury (Wallace _et al_., 1971).

Of particular significance with regard to assessing the potential health hazard of mercury is the fact that the mercury in fresh water fish flesh is predominantly in the form of methylmercury compounds, despite the fact that most mercury released into rivers, lakes, and oceans is in the form of the inorganic salt or the metallic element (Goldwater and Clarkson, 1972). Methylmercury becomes available in the fish food chain through the transformation of inorganic mercury into the organic methylmercury form by microorganisms or other biologically derived alkylating systems present in the sediments of lakes, rivers, and estuaries. These systems are capable of forming methylmercury and dimethylmercury from inorganic mercury, under both aerobic and anaerobic conditions.

Although environmental mercury had been a matter of concern and under intensive investigation for many years elsewhere, notably in Sweden and Japan, it was not until 1970 that the problem received noteworthy attention in the United States. In March 1970, a Norwegian investigator working in Canada reported high concentrations of mercury in fish from Lake St. Clair. This triggered extensive investigations of mercury in fish from both Canada and the United States (Goldwater, 1971). The U.S. FDA established a mercury concentration of 0.5 ppm in fish tissue as a guideline for evaluating results of the fish investigations. By September 1970, 18 states had taken specific actions, ranging from general warnings to closure of fishing in designated waters. Attention was focused on mercury-containing waste discharges from chlor-alkali plants. The operators of these plants took prompt action to reduce mercury discharges. The reductions were monitored by the Department of the Interior, which found that the overall extent of mercury emission dropped 86%, from 287 lb/day in July to 40 lb/day in September (Wallace _et al_., 1971).

Chemical Characteristics. Metallic mercury is regarded as virtually insoluble in water. Mercury forms two series of salts, traditionally considered as being univalent and divalent. However, it has been shown that the "univalent" compounds contain the group Hg_2^{+2} (or $^+Hg-Hg^+$), with two mercury atoms covalently bound to each other, so this series is actually divalent. Univalent (mercurous) salts are mostly insoluble, and the divalent (mercuric) series is mostly soluble, except the iodide and sulfide. Mercury has a remarkable ability among metals to form compounds with organic radicals, normally linking covalently to a carbon atom. Organic mercury compounds can be conveniently classified into two types, RHgX and R_2Hg, where R is an organic radical and X an inorganic (radical) ion. RHgX compounds in general are crystalline solids whose properties depend on the nature of X. When X is chloride, bromide, iodide, cyanide, thiocyanide, or hydroxyl, the compound is a covalent nonpolar substance more soluble in organic liquids than in water. When X is a sulfate, nitrate, phosphate, or perchlorate radical, the substance is salt-like, that is, ionic. R_2Hg compounds are nonpolar, volatile, toxic liquids or low-melting-point solids. All are thermally unstable and light sensitive. (Wallace _et al._, 1971.)

Metabolism. Investigations of the metabolism of mercury and its various compounds, particularly the comprehensive studies in Japan and Sweden, have been reported on extensively. Takeuchi (1970) has summarized Japanese investigations of the biologic reactions and pathologic changes in human beings and animals caused by organic mercury contamination.

Specific data on human excretion of methylmercuric nitrate were derived from studies with orally administered labeled compound. Three male volunteers were given an oral dose of [^{203}Hg] methylmercuric nitrate. Over 90% was absorbed; maximum blood content was reached 3-6 h after ingestion. The liver contained 55% of the total radioactivity, with 12% in the head. The biologic half-life was determined to be 70-74 days (Ekman _et al._, 1968; Aberg _et al._, 1969; Falk _et al._, 1971).

Methylmercury and other short-chain alkylmercury compounds exert their main toxicologic effects on the nervous system. In man, methylmercury concentrations in blood cells and hair provide the best index of exposure of the nervous system to methylmercury compounds. If exposure to other mercury compounds is minor, compared with exposure to methylmercury, analysis of total mercury may be used instead. Blood concentrations of mercury reflect more accurately the intake from recent exposure to methylmercury; hair concentrations reflect the average intake over a long period. The mercury concentrations in successive segments of hair over the period of its formation can indicate the degrees of past absorption of mercury compounds.

The factors that determine the biotransformation of mercurials, their passage through barriers in the body, and the ultimate action on cellular mechanisms are only beginning to be understood. The amount of a particular compound present in the body is the result of a balance between intake and excretion. When the same amount is taken in each day, the body content rises progressively to a plateau at which excretion equals intake. The time to reach a steady state in the body is determined by the half-time of excretion. Taking the half-time of excretion in man as 70 days, a steady state in a person will be reached in approximately 1 year. Once attained, the steady state concentration of mercury is proportional to the daily intake.

studies of methylmercury in humans support this conclusion (Goldwater *et al.*, 1972).

Health Effects. Exposure to metallic mercury via routes other than inhalation is infrequent. Oral doses of 100-500 g have been given to man with little effect, because of poor absorption, although they occasionally resulted in diarrhea. The comparative toxicity of inorganic mercury salts is related to their absorption. Thus, insoluble mercurous salts, such as calomel (mercurous chloride), are relatively nontoxic. In man, some data are available on accidental or intentional overdosage with mercuric chloride. The immediate effects of acute poisoning are due to irritation, coagulation, and superficial corrosion of exposed tissues. Chronic effects include kidney damage, as intestinal hemorrhage, and ulceration. Investigation of laboratory technicians subject to inhalation exposure to mercuric chloride (0.2-0.3 mg/m³) showed high urinary concentrations of protein, considered suggestive of early renal tubular dysfunction. From these considerations, it appears that metallic mercury and inorganic mercury salts themselves are not significant contributors to the current problem concerning environmental contamination. The problem appears to be related mainly to methylmercury compounds in the environment, particularly in fish, and to accidental ingestion, either of treated seed grain or of meat from animals that had been fed grain treated with alkylmercury compounds (Lu *et al.*, 1972).

Two major outbreaks of environmentally related methylmercury intoxication have occurred in recent years in Japan--in the Minamata Bay area (1953-1961) and in Niigata (1964-1965). Of the 121 cases recorded in the Minamata Bay episode, there were 46 deaths. About half the adult victims, one-third of the children, and one-eighth of the fetal victims died. In Niigata, 30 cases, including five deaths, were reported in 1965. Thereafter, 17 cases, including one death and one fetal case, were reported from 1966-1970. In both outbreaks, industrial pollution of waters, with later contamination of fish and shellfish by mercury as methylmercury, was shown to be the cause. (Berglund, 1971). There was no indication in the report that drinking water was considered as a possible contributor to the outbreaks.

There have been many reports of poisoning after accidental ingestion of methylmercury compounds. In Pakistan, in 1961, several families became ill after eating wheat seed treated with phenylmercuric acetate and ethylmercuric chloride, and 5 of 14 hospitalized patients died. A similar episode occurred in Guatemala in 1965, when there were 20 deaths among 45 people who displayed typical symptoms of mercury poisoning after eating wheat seed treated with methylmercury dicyandiamide. In Iraq, 331 cases of poisoning with 36 fatalities resulted between 1956 and 1960, owing to ingestion of seed treated with ethylmercuric-p-tolylsulfanilide. In Ghana, in 1967, 17 of 65 persons died after ingestion of stolen maize that had been treated with Merkuran, a product containing 2% ethylmercuric chloride (Lu *et al.*, 1972). Members of a family in Alamogordo, New Mexico, were victims of mercury intoxication in 1969 from eating meat from animals that had been fed grain treated with alkylmercury compounds. This episode is particularly significant, because it demonstrated the effects of methylmercury on the fetal nervous system: There was a case of mercury poisoning during a pregnancy. The patient's mother ate mercury-contaminated pork, probably during the second trimester of her pregnancy. The full-term boy who was born had severe tremors at birth, which persisted for several days. His urinary mercury concentration during the first day of life was 2.7 ppm, 100 times the quoted normal adult concentration. By 6 weeks of age, the infant was noted to be hypertonic and irritable; mercury could not be detected in his urine. By 8 months of age, he had myoclonic seizures and was hypotonic, irritable, grossly retarded, and probably cortically blind. He had never been breast fed; this fact provided evidence that this was a case of intrauterine poisoning. The mother was asymptomatic, despite having documented increased urinary mercury concentrations during the third trimester of her pregnancy (Scanlon, 1975).

The frequency of occurrence of various symptoms and signs in Minamata disease (methylmercury poisoning) among adults, children, and infants as recorded in the Japanese studies is presented in Table V-11.

TABLE V-11
Frequency of Occurrence of Various Symptoms and Signs in Minamata Disease (%)

Symptoms	Fetal	Children	Adults
Mental disturbance	100	100	71
Ataxia	100	100	94
Impairment of the gait	100	100	82
Disturbance in speech	100	98	88
Hearing impairment	4.5	67	85
Constriction of visual fields		100?	100
Disturbance in chewing and swallowing	100	89	94
Brisk and increased tendon reflex	82	72	38
Pathological reflex	54	50	12
Involuntary movement	73	40	27-76
Primitive reflex	73	0	0
Impairment of superficial sensation	?	?	100
Salivation	72	56	24
Forced laughing	27	29	

(From Takeuchi, 1970)

The amount of methylmercury needed to produce Minamata disease is not known, nor is there a specific biochemical test available as a diagnostic aid in mercury poisoning (Goldwater *et al.*, 1972). Establishment of exact relationships between the dose of a mercurial taken into the body and the health effects expected presents a number of difficulties. The nature of the mercury compound has a marked effect on absorption and metabolism, and therefore on toxicity. Data that refer only to the amount of elemental mercury are not of much help. Methods of analysis have not been very sensitive, so the effects of small doses continued over long periods cannot be followed accurately.

In a report issued in 1970, the Swedish Commission on Evaluating the Toxicity of Mercury in Fish set forth its recommendation related to allowable intakes of methylmercury (Berglund, 1971). It recommended the use of the "allowable daily intake" (ADI) as a method of warning consumers so they could restrict their intake of mercury-contaminated foods. On the basis of available information, they concluded that it appeared "justifiable" to assume that clinically manifest poisoning of adults sensitive to methylmercury may occur with a concentration in whole blood down to 0.2 µg/g, which seems to be reached on exposure to about 0.3 mg of mercury (as methylmercury) per day, or about 4 µg/kg of body weight per day. It was pointed out, however, that concentrations of 0.2 µg/g or higher in the blood cells had been measured in some 20 persons and concentrations exceeding 0.8 µg/g in four persons without any clinical symptoms of methylmercury poisoning in Sweden and Finland, and even concentrations of 50 µg/g or more in the hair of at least 130 persons in Japan who were not considered to be poisoned. They cited a number of elements of uncertainty that must be considered in further evaluation of these conclusions, including the acknowledgment that the data on prenatal poisoning are particularly limited. A safety factor of 10 was applied to the lowest mercury exposure that was assumed to cause the neurologic symptoms of clinically manifest intoxication. It was concluded that the "acceptable daily intake" of methylmercury through fish would correspond to about 0.03 mg of mercury (as methylmercury), or about 0.4 µg/kg of body weight.

Analysis. Mercury in fresh water is below the detection limit of 250 µg/liter by conventional flame atomization. Issaq and Zielinski (1974) observed a 50-fold mercury signal enhancement when hydrogen peroxide was added to the aqueous mercury solution. Solvent extraction with APDC or dithizone may be used, but the preferred method of analysis is the cold-vapor technique of Hatch and Ott (1968). By this procedure, mercury vapor is formed by reduction of mercuric ions in solution by stannous chloride and passed through an absorption cell situated in the light path of a spectrophotometer. Detection limits better than 0.1 μg/liter are easily obtainable for water samples. Most other published procedures also use stannous chloride as the reducing agent (U.S. EPA, 1971; Omang, 1971; Traversy, 1971; Baltisberger and Knudson, 1974).

Mercury in organic matter can be oxidized by persulfate; the resulting solution is analyzed for mercury by flameless atomic absorption (Alberts *et al.*, 1974; Feldman, 1974).

Methylmercury can be extracted with benzene and then subjected to flameless atomic-absorption analysis (Bisogni and Lawrence, 1974).

Conclusions And Recommendations. The current problems concerning mercury contamination of the environment appear to be related mainly to methylmercury compounds. As far as humans are concerned, the presence of these compounds in foods (principally fish) and the accidental ingestion of treated seed grain or the ingestion of meat from animals that had been fed grain treated with alkylmercury compounds are the major problems. Drastic limitations imposed or being imposed by official agencies on the industrial discharge of mercury-containing wastes that contribute to methylmercury contamination of fish, on the allowable mercury content in fish used for human consumption, and on the use of mercurial fungicides should minimize the mercury hazard to man from these sources. There is no indication that mercury compounds in the concentrations and forms found in the ambient atmosphere or in drinking-water supplies contribute significantly to methylmercury intoxication in humans.

European drinking-water standards (WHO, 1970) do not contain a standard for mercury. The international standards for drinking water (WHO, 1971) recommend that the tentative upper limit for mercury in drinking water be 1 μg/liter. The U.S.S.R. has a standard of 5 μg/liter (inorganic compounds only) (Stofen, 1973). The U.S. EPA-proposed drinking-water standard for mercury is 2 μg/liter (total mercury). Drinking water containing mercury at this concentration will contribute a total of 4 μg to the daily intake. According to the EPA, only a small fraction of the mercury in drinking water is in alkyl form, and the contribution of methylmercury to the daily intake will be less than 4 ng (0.004 μg)--approximately 0.01% of the ADI for methylmercury recommended by the Swedish commission. At this level, the potential hazard to humans from mercury in U.S. water supplies is inconsequential, compared with the contribution from food.

In light of this and the fact that nearly all drinking-water supplies in the United States are already in compliance with the current interim regulation, there is serious question as to whether a standard is needed or serves any useful purpose. There is however, a lack of firm data on the ratio of organic mercury to total mercury in drinking-water supplies, although it is generally accepted that mercury in drinking water is principally in inorganic form. Until it is demonstrated that this belief is universally applicable to water supplies, it appears desirable to limit the concentration of mercury in drinking water as if it were methylmercury.

There is a need for specific investigations to validate or modify the prevailing opinion that mercury in drinking water is principally in the inorganic form.

Molybdenum

Occurrence. Molybdenum metal and its salts are used primarily in metallurgy and for electric and electronic apparatus. Other uses are in the glass and ceramics industries, for the production of pigments, and as a constituent of fertilizers for leguminous crops (McKee and Wolf, 1963; NAS, 1973; Davis, 1974). Molybdenum salts can reach surface and ground water as a result of the mining of molybdenum sulfide. They are also byproducts of the mining and milling of uranium. The burning of fossil fuels and natural weathering processes are other sources of molybdenum in the environment. Transport can be by air and water (Chappell, 1973).

Molybdenum is present in surface water and ground water at very low concentrations. With emission spectroscopy, Durum and Haffty (1961) measured molybdenum in 59 samples of surface water from 15 rivers in the United States and Canada. The maximum concentration observed was 6.9 µg/liter, in the Colorado River at Yuma, Arizona. Kopp and Kroner (1967) noted the presence of molybdenum in 32.7% of their surface-water samples from the 15 major river basins of the conterminous United States, with concentrations ranging from 2.0-1,500 µg/liter. The overall mean was 60 µg/liter, and 26 stations had means greater than 50 µg/liter. Of the four stations recording the highest values, three were in Colorado and one was just across the border on a stream draining from Colorado (Chappell, 1973).

Chappell (1973) reported tap-water concentrations as high as 580 µg/liter. In the finished-water supplies of the 100 largest cities in the United States, Durfor and Becker (1964) reported the maximum molybdenum concentration as 68 µg/liter, the median as 1.4 µg/liter, and the minimal as not detectable. In a survey of 380 finished waters in the United States between 1 October 1962 and 30 September 1967, Kopp (1973) reported that 29.9% had measurable concentrations of molybdenum, with a maximum of 1,024 µg/liter, a minimum of 3 µg/liter, and a mean of 85.9 µg/liter. According to Hadjimarkos (1967), the mean drinking-water concentration was 8.0 µg/liter. Kehoe *et al.* (1944) reported that the concentrations of molybdenum ranged from nil to 270 µg/liter in ground water and from 0.1-0.5 µg/liter in sea water. Wells used for watering livestock and irrigated forage at Canon City, Colorado, had up to 25,000 µg/liter and resulted in molybdenosis (Chappell, 1973).

Barnett *et al.* (1969) studied several trace metals in raw, treated, and tap water for the Denver municipal system. This system draws its supplies from three watersheds (one being Dillon Reservoir) and treats them in four filter plants, one of which is an old, slow sand filter. Distributed water was sampled at four carefully selected locations in residences. On 15 September 1966, water from Dillon Reservoir reached 330 μg/liter of molybdenum, but it was blended with water from the South Platte River before filtration.

Unlike copper and manganese, molybdenum is not removed significantly by treatment processes and not changed by distribution. At one tap, for example, a sample collected in May 1966 (when very little water from Dillon Reservoir was being used) contained 8 μg/liter; by 16 September 1966, the concentration in water from this tap was 190 μg/liter. Distribution and plumbing have very little, if any, measurable effect on molybdenum concentration.

The U.S.S.R. has established a limit of 0.5 mg/liter for Mo^{+6} in surface water (Stoefen, 1973). Konovalov et al. (1966) reported the concentration of suspended and dissolved molybdenum in four major drainage basins of European U.S.S.R. In 32 samples, molybdenum was detected in only four, with a maximum concentration of 14.3 μg/liter and a minimal concentration of 0.1 μg/liter. High concentrations occurred in rivers draining molybdenum mining and milling operations.

The atmospheric transport of molybdenum may be significant, but human ingestion of airborne molybdenum is unlikely to constitute a major pathway of intake. Kaakinen and Jorden (in Chappell, 1973) studied the fate of molybdenum in a coal-fired electric power plant in Colorado. Their results showed that molybdenum from coal is definitely enriched in fly ash leaving a coal-fired power plant in stack gases (even after treatment with electrostatic precipitators and wet scrubbers), but significantly decreased in bottom ash. Enrichment in fly ash appears to be related to the volatility of molybdenum and its adsorption on fine particles. Molybdenum that escapes in particulate matter in stack gases may be expected to settle to earth and enrich the soil, plants, and water, thereby possibly contributing to molybdenosis of livestock.

It is concluded that molybdenum in drinking water, except possibly from highly contaminated molybdenum mining wastewater, is not likely to constitute a significant portion of the total human daily intake of molybdenum. For example, according to Hadjimarkos (1967), the average drinking water provides only 1.6% of the daily human intake of molybdenum.

Chemical Characteristics. Molybdenum occurs in nature in a IV oxidation state in the sulfide molybdenite (the commercial source) and in a VI oxidation state in molybdate salts. It is uniformly distributed among igneous rocks, with a slight concentration in basaltic rocks. It makes up approximately 2 mg/kg of the continental crust (Davis, 1974). About 60% of the molybdenum mined in the United States is taken from the world's largest deposit of molybdenum sulfide, near Climax, Colorado (Chappell, 1973).

According to Amanguiyan (1965), molybdenum sulfide is sparingly soluble in water, but is fairly readily oxidized to form more soluble molydates (salts of molybdic acid), which are stable in water in the absence of a reducing agent. Organoleptic tests showed that ammonium molybdate imparted a slightly astringent taste to water, starting at a molybdenum concentration of 10 mg/liter. A concentration of 100 mg/liter produced a marked inhibitory effect on the biochemical oxygen demand (BOD) of water, but 10 mg/liter had no effect on the total number of bacteria in water.

Metabolism. Schroeder _et al._ (1970) estimated that the daily intake of molybdenum was 390 µg. Hadjimarkos (1967) estimated the human intake of molybdenum in food at 1,000 µg/day. On the basis of very limited data, Tipton _et al._ (1966) calculated the total dietary intake of molybdenum by adults to be approximately 100 µg/day.

The mean daily dietary intake of molybdenum is so small (about 1.0 mg/day) and of such minor importance that its role in human nutrition and metabolism has been studied very little (Davis, 1974). Miller _et al._ (1959) did balance studies on 24 girls, 7-9 years old, who were fed molybdenum at an average of 75 mg/day; most of this appeared in the urine. The higher the protein intake, the less molybdenum was retained.

Molybdenum concentrations are normally very low in animal tissues. Davis (1974) presented the following concentrations for adult man:

Tissue	Molybdenum concentration, mg/kg (dry weight)
liver	3.2
kidney	1.6
spleen	0.20
lung	0.15
brain	0.15
muscle	0.14

The molybdenum content of food varies greatly. In general, legumes, cereal grains, leafy vegetables, liver, and kidney are good sources; fruits, root and stem vegetables, muscle meats, and dairy products are among the poorest.

Molybdenum is excreted primarily in the urine, probably as molybdate anion (Davis, 1974).

According to Aemanguiyan (1965), molybdenum is fairly rapidly eliminated from animals, but it has some slight cumulative properties, especially in the bones, kidneys, and liver.

Health Effects. Molybdenum is recognized as an essential mineral for both man and other animals. It is an integral part of at least two mammalian enzymes, xanthine oxidase and aldehyde oxidase (USFDA, 1975).

In man, molybdenum poisoning has rarely been observed. High intakes in Armenia (USSR) have been associated with high incidences of gout. In India, a bone-crippling disease occurs in areas where sorghum contains high amounts of molybdenum; it has been postulated that molybdenum increases the toxicity of fluoride in producing this disease. Both these human-related incidents are speculative and await more definitive information to establish cause-and-effect relationships (USFDA, 1975).

Although it is known that molybdenum is an essential trace element (Browning, 1961), excessive dosages in laboratory animals and in the forage of herbivores, such as cattle, have been deleterious. Molybdenum is picked up by forage crops from the soil moisture and concentrated in the foliage. Water-soluble molybdates in herbage cause cattle to scour severely--i.e., to have diarrhea--which sometimes results in death. Soil moisture from pastures in which cattle had scoured contained molybdenum at 20-100 mg/liter, whereas noninjurious fields contained less than 5 mg/liter. The herbage content of molybdenum varied with plant species on the same soil (McKee and Wolf, 1963, citing several references).

Animals vary greatly in their sensitivity to molybdenum. Cattle appear to be the most sensitive, with severe diarrhea occurring at intakes as low as about 20-100 mg/kg of forage. Pigs tolerate 1,000 mg/kg of diet without discernible ill effects. In young chickens, dietary supplements of 200-2,000 and 4,000 mg/kg of food induced growth suppression and anemia. In rats being fed a copper-deficient diet, molybdenum at 10 mg/kg of food produced a reduction in the rate of weight gain, but if copper at 1 mg/kg of diet was present, no effect was observed when molybdenum was added at 100 mg/kg. High sulfate or sulfate-forming components, such as methionine, exacerbated the molybdenum toxicity (USFDA, 1975).

The toxicity of molybdenum in animals is related to a number of dietary factors, including copper, sulfate, endogenous sulfate-producing substances, and other trace metals that affect copper metabolism. The mechanisms by which these components ameliorate or intensify molybdenum toxicity are largely unknown, owing to the complexity of the interrelationships of these nutrients. Signs of toxicity in most species are similar to those of copper deficiency and often include reduced growth, loss of appetite, anemia, hair loss, bone defects, and loss of hair color (USFDA, 1975).

The daily food intake of animals is appreciably lower on molybdenum-containing diets than on control diets. Rejection of the diet may be a conditioned response caused by sensory detection of the presence of molybdate ion or its interaction with unidentified constituents of the diet (Monty, 1960).

Rats fed toxic dosages of molybdate showed a reduction in liver sulfide oxidase activity. Significantly, rats were unable to discriminate between the molybdate-containing diet and the control diet for the first 5 days after molybdate was added to the diet. If diets that had been "aged" for at least 5 days were offered to rats, they were able to discriminate against the molybdate-containing diet within 2 days. It has been postulated that molybdate interacts with some dietary constituent over a period of 5 days to produce a change that permits the rats to discriminate (Anon., 1962).

According to Asmangulyan (1965), who introduced various doses of molybdenum into the diets of young rabbits, chronic poisoning by molybdenum gives rise to marked functional changes, including an increase of sulfhydryl groups in the serum and liver and a decrease in vitamin C in the liver, at dosages as low as 0.5 mg/kg of diet. The fact that a rise in the content of biologically active sulfhydryl groups has been shown to be caused by molybdenum may assist in elucidating the mechanism of the "molybdenum gout" that is found in Armenia. The inactive dose of molybdenum for rabbits was 0.025 mg/kg of diet (or approximately 0.5 mg/liter in drinking water). As a result, Asmangulyan recommended a maximum permissible concentration of molybdenum in open bodies of water of 0.5 mg/liter. This recommendation may well have been the evidence on which the U.S.S.R. limit was based.

Analysis. With atomic-absorption spectrophotometry, a detection limit of 20 μg/liter is attainable by direct aspiration into the flame, necessitating concentration for ordinary determinations. Chau and Lum-Shue-Chan (1969) have studied extraction systems for molybdenum and recommended an oxine-methylisobutylketone system, with extraction at a pH of 2-2.4. When the graphite furnace is used to increase sample atomization the detection limit is lowered to 0.5 μg/liter.

Conclusions And Recommendations. Soluble molybdate ions are present in trace concentrations in many surface waters, primarily as a result of industrial waste, but also as a product of natural weathering of molybdenum-bearing soils. Both suspended insoluble molybdenum sulfide and soluble molybdates are present in streams that drain areas where molybdenum ore is mined and processed, especially in Colorado and New Mexico.

Typical diets contain molybdenum at around 100-1,000 µg/day, whereas typical surface water (except that draining mining areas) contains nil to about 100 µg/liter, with mean or median of about 10 µg/liter. Hence, it is evident that water is a minor factor in the total molybdenum intake in most locations.

In humans, molybdenum poisoning has rarely been observed. Although it has been implicated in gout in Armenia and in a bone-crippling disease in India, these involvements are speculative and await more definitive information to establish cause-and-effect relationships.

Molybdenosis in livestock, however, is a significant toxicologic problem in many areas. Consumption of molybdenum-rich forage by cattle and sheep causes severe diarrhea (scouring) that sometimes results in death. It can be prevented or ameliorated by the administration of copper, but the relationship of molybdenum, copper, and sulfate-forming compounds in animal metabolism needs further study.

The U.S.S.R. has established a molybdenum limit of 0.5 mg/liter in open water, but the WHO has not yet promulgated a limit (Stoefen, 1973). The *National Primary Drinking Water Regulations* USEPA, 1975) do not set any limit for molybdenum.

Nickel

The National Academy of Sciences has recently completed an extensive review of the medical and biologic effects of nickel (1975). This section includes excerpts from that publication (with appropriate page numbers shown).

> Occurrence. Kopp and Kroner reported that nickel was found in U.S. waters with a frequency of 16% and at an overall mean concentration of 19 µg/liter. The detection limit for nickel in water with total dissolved solids of 400 µg/liter was 20 µg/liter. If the dissolved solids amounted to 200 µg/liter, the detection limit would be 10 µg/liter (p. 9).
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> The Missouri River and Western Gulf basins had the lowest frequency of nickel detection and among the lowest mean concentrations, at 5 and 3 µg/liter, respectively. The highest mean concentration was 130 µg/liter, in the Cuyahoga River at Cleveland, Ohio (p. 9).

It was concluded that most of the nickel in surface water and ground water originates from man's activities. This conclusion was strengthened by data on nickel concentrations determined by spectrographic analysis of evaporated residue of selected samples taken in 1962 of public water supplies of the 100 largest cities in the United States.

> On the basis of analyses of nickel concentrations of 969 water supplies in the United States during 1969-1970,... the average concentration of nickel in water samples taken at the consumer's tap was 4.8 µg/liter. With an estimated daily intake of 2 liters of water, an adult would consume approximately 10 µg of nickel per day in drinking water (p. 11).
>
> Man's exposure to nickel in food derives from the natural occurrence of nickel in food ingredients and from man-made sources, such as alloys, food-processing equipment, and fungicides, which may increase the amount of nickel in food substances beyond that naturally present.... With the exception of some preliminary studies in plants, nothing is known about the chemical form of nickel in foods. Detailed information of this type needs to be developed for consideration of possible differences in bioavailability and biotoxicity of nickel in foods. However, the available information indicates that the concentrations of nickel in foods are low and do not pose any toxicity problem (p. 51).
>
> The usual oral intake of nickel by American adults (has been calculated) at 300-600 µg/day. Nickel ingestion may vary

widely. (It has been calculated) that a person who ingests a 2,300-cal diet containing 100 g of protein, 250 g of carbohydrates, and 100 g of fat and who consumes meat, milk, fruit, refined white bread, wheatena, butter, and corn oil would take in 3-10 µg of nickel per day. At the other extreme, a diet that has the same calorie value and the same proportions of protein, carbohydrate, and fat might contain 700-900 µg of nickel per day, if the person consumes oysters, meat, milk, eggs, oats, whole-wheat or rye bread, some vegetables, potatoes, and legumes, with little added fat. The wide range of oral intake of nickel may also result from variable ingestion of beverages--such as tea, coffee, beer, and red wine--that contain more than 100 µg of nickel per 100 g (p. 62-63).

Chemical Characteristics. With the exception of carbonates, rocks low in silica are high in nickel, and those high in silica are relatively low in nickel. Farm soils of the world contain nickel at 0.0003-0.1%. The average farm soil in the United States contains nickel at more than 0.003%. Soils with less than 0.0003% are too acidic to support normal plant growth (p. 5).

The nickel content of seawater ranges from 0.1-0.5 µg/liter. In most groundwaters, nickel has not been identified; and in instances where it has been detected, analysts theorize that it is probably in colloidal form (p. 8).

It has been determined that, in the rock-weathering process, nickel goes into the insoluble minerals of the hydrolysates. Therefore, any nickel in surface or groundwaters is likely to be in small amounts, unless its presence is due to industrial pollution (p. 8).

Metabolism. Most of the nickel that is ingested in food remains unabsorbed within the gastrointestinal tract and is excreted in the feces.... Fecal excretion of nickel by healthy human subjects (has been reported to be) 100 times greater than urinary excretion.... There appears to be a mechanism that limits the intestinal absorption of nickel in mammals, despite the relatively large amount of nickel present in their food (p. 63).

Inhalation from the atmosphere and tobacco smoke provides a mode of entry of nickel into the body:

The reported mean nickel content of cigarettes have ranged from 2.0-6.2 µg/cigarette. Analyses... have shown that 10-20% of the nickel in cigarettes is released into the mainstream smoke. Of that nickel 84% is in the gaseous phase and only 16% in the particulate phase. [There is suggestive evidence] that gaseous nickel in mainstream smoke occurs in the form of nickel carbonyl (pp. 178-179).

[It has been] calculated that a cigarette smoker would inhale a maximum of 14.8 µg of nickel per day from 40 cigarettes. [The estimated] actual retention of inhaled nickel within the body is probably only 75% of the calculated intake... (pp. 63-64).

There is wide variation in the average concentrations of nickel in urban atmospheres. Of urban areas of the United States that were surveyed during 1964 and 1966, the cleanest with respect to atmospheric nickel were Boise, Idaho; Albuquerque, New Mexico; and Moorhead, Minnesota. No nickel was detected in those three areas.... In comparison, the cities with the highest atmospheric concentrations of nickel were New York City (1966 average, 0.118 µg/m³ of air) and East Chicago, Indiana (1964 average, 0.69 µg/m³).... The daily inhalation of nickel by residents of New York City and East Chicago [was estimated], assuming that 20 m³ of air (24.1 kg) is inhaled daily,...at 2.36 µg of nickel per day and...13.8 µg of nickel per day [for the two cities, respectively] (p. 63).

The metabolism of nickel that enters the body by the pulmonary route is similar to that of nickel compounds that are administered parenterally. Inhaled nickel carbonyl is excreted primarily in the urine and to a minor degree in the feces.... A correlation of atmospheric concentrations of nickel in a nickel smelting plant with the concentrations of nickel in the urine of exposed workmen [has been rep[illegible] (pp. 64-65).

It has also been shown that measurements of nickel in serum and urine can serve as biologic indexes of environmental exposure to nickel.

Health Effects. Man is not naturally exposed to the inhalation of atmospheric nickel, with the possible exception of nickel from volcanic emanations. The available evidence indicates that the natural concentrations of nickel in waters, soils, and foods do not constitute a biologic threat. Indeed, nickel may be an essential trace element for the nutrition of man and animals (p. 191).

Recent evidence suggests that nickel partially satisfies the criteria for essentiality of trace elements as micronutrients: presence of the element in the fetus or newborn, presence of homeostatic regulation of the metabolism of the element, demonstration of a metabolic pool of the element that is specifically influenced by hormonal substances or pathologic processes, demonstration of a metalloenzyme of which the element is an integral part, and demonstration of a deficiency syndrome that can be prevented or cured by trace amounts of the element (p. 89).

Nickel is probably essential for animal nutrition, but there has not yet been unequivocal demonstration that nickel deprivation produces consistent abnormalities in experimental animals that can be prevented or cured by the administration of nickel.

Toxicity studies have demonstrated that nickel and nickel salts have relatively low toxicity in various species of animals when administered orally. However, parenteral injections of nickel salts are much more toxic. Major signs of acute nickel toxicity consist of hyperglycemia and gastrointestinal and central nervous system effects. Ingested nickel is excreted primarily in the feces, whereas parenterally administered nickel is excreted mostly in the urine. Little informaion is available on animals relative to the acute effects of inhaled nickel compounds, except for nickel carbonyl, which is extraordinarily toxic.... Several nickel-containing substances--including nickel dust, nickel subsulfide, nickel oxide, nickel carbonyl, and nickel biscyclopentadiene--have been demonstrated to be carcinogenic in experimental animals after inhalation or parenteral administraion. There is no evidence that nickel compounds are carcinogenic in animals after oral or cutaneous exposure. There is very little information on the teratogenicity or mutagenicity of nickel compounds in experimental animals (p. 192).

Epidemiologic studies of workmen in nickel smelters and refineries have revealed a significantly increased incidence of cancers of the lungs and nasal cavities.... Respiratory cancers in nickel workers have usually developed after long latent periods, such as are typical of occupational cancers.... There is only scanty evidence of an increased incidence of respiratory cancers among workmen who have other types of occupational exposure to nickel, such as nickel electroplating and grinding. (pp. 192-193).

Nickel is a common cause of chronic dermatitis in man, as a result of industrial and other exposures [and] use of nickel-containing alloys in jewelry, coinage, clothing fasteners,... utensils, [and] implanted therapeutic devices and prostheses...(p. 193).

Berg and Burbank (1972) found nickel in drinking water to be poorly correlated with mortality from oral or intestinal cancer. There was no correlation between nickel and mortality from nasal or pulmonary cancer, even though these are the types of cancer usually associated with industrial exposure to nickel.

Analysis. Conventional flame atomization has a nickel detection limit of 2 µg/liter. Extraction procedures are usually used to concentrate the nickel before analysis. The APDC-methylisobutylketone extraction at a pH of 2.8 is used by the U.S. Geological Survey (Brown *et al.*, 1970). Others have used similar procedures for fresh-water analysis (U.S. EPA, 1971; Paus, 1971; Traversy, 1971; Kinrade and Van Loon, 1974; Aldous, 1975). Jenne and Ball (1972) have studied the stability of the nickel-APDC complex. Diethyldithiocarbamate has also been used as a chelating agent (Joyner *et al.*, 1967; Nix and Goodwin, 1970). Paus (1971) and Surles *et al.* (1975) have used the graphite furnace to increase sample atomization for fresh-water analysis; this procedure has a nickel detection limit of 1 µg/liter with direct sampling into the furnace.

Conclusions and Recommendations. Because of the low toxicity of nickel and nickel compounds in food and drinking water, the low concentrations present in drinking water, and the small daily intake of nickel in drinking water (compared with food), there is no present need to establish nationwide limits for nickel in drinking water. The U.S. EPA *National Interim Primary Drinking Water Standards* and the WHO European Standards for drinking water do not include standards for nickel.

There is no pressing need for research with regard to nickel in drinking water. In this regard, however, research to clarify the role of nickel in nutrition appears to be desirable, particularly as to its dietary essentiality.

Silver

Occurrence. The intentional addition of silver to drinking water for disinfection is one possible source of silver in public water supplies. The silver ion has bactericidal characteristics at concentrations of 15-50 µg/liter (Von Nageli, 1893). As a result the Katadyn process and others have been promoted for treatment of drinking and swimming-pool water. The bactericidal action is slow, especially in cold water, and silver is neither viricidal nor cysticidal in the concentration used (Renn *et al.*, 1955). Dosages in excess of 150 µg/liter have been used in swimming pools, but, because of cost and the opalescence caused by colloidal silver chloride, the method is not practical or recommended for public supplies.

Data from 1,577 samples of well and surface water from 130 points in the United States showed detectable concentrations (0.1 µg/liter or more) of silver in only 104 samples. The concentration ranged from 0.1-38 µg/liter, with a median of 2.6 µg/liter (Kopp, 1969). The highest concentrations were noted in the St. Lawrence and Colorado Rivers (Durum and Haffty, 1961).

The examination of finished water in public supplies of the 100 largest cities in the United States revealed trace quantities of silver as high as 7 µg/liter, with a median of 2.3 µg/liter (Durfor and Becker, 1964). Another survey of finished water found silver in 6.1% of 380 sample, with concentrations of 0.3-5 µg/liter (mean, 2.2 µg/liter) (Kopp, 1973). Chemical analysis of *Interstate Carrier Water Supply Systems* (U.S. EPA, 1975) reported nondetectable silver (<0.1 µg/liter) in 45% of the analyses; 99.5% of all determinations were equal to or less than 50 µg/liter, the interim standard. The community water-supply survey (McCabe, 1970) found that none of the 2,595 samples from household taps exceeded the standard. The maximum concentration was 30 µg/liter.

Unless lime softening in the water-treatment process results in a high pH, very little difference in silver concentration may be expected between raw and finished water. When water containing silver is used for culinary purposes, it is reasonable to assume that vegetables belonging to the family Brassicaceae - such as cabbage, turnips, cauliflower, and onions - would combine with the residual silver in the cooking water. The silver content of 2 or 4 liters of water could thus be ingested, but rarely by one person. Soil contains only small amounts, but humus from decaying plants may contain up to 5 ppm. Some foods, such as bran and wheat flour, contain trace quantities (less than 1 ppm), but mushrooms have unusually high concentrations - up to several hundred parts per million (Ramage, 1930).

Chemical Characteristics. Trace amounts of silver are found in natural and finished water originating from natural sources and from industrial waste. Silver is a rather rare element with a low solubility of 0.1-10 mg/liter, depending on pH and chloride concentration (Hem, 1970). Water-soluble silver compounds include the acetate, chlorate, nitrate and sulfate.

Metabolism. For reasons still unknown, individuals and individual organs absorb silver selectively. Tissues of animals and humans do not often contain silver. About 10% of tissues and samples contain silver, and the concentrations rarely exceed 0.01 mg/100 grams (Kehoe et al., 1940). The cases of generalized argyria prove that silver can be absorbed from the gastrointestinal tract, by inhalation of dust, and after medication with silver compounds.

Excretion of silver is almost entirely in the feces, with only a trace to be found in the urine. There is little retention of silver in general, but, when it occurs the greatest concentrations are found in the reticuloendothelial organs. After intravenous injection in animals (Gammill, 1950), the order of silver concentration is spleen, liver, bone marrow, lungs, muscle, and skin. The balance between intake and elimination is inconclusive, but evidence suggests that ingested silver is only slightly stored.

Health Effects. Large single doses of colloidal silver can be fatal. A dose of 500 mg was lethal in a dog in 12 h (Shouse and Whipple, 1931); death was due to pulmonary edema and was preceded by anorexia and anemia.

In addition to hyperplasia of the bone marrow, repeated injection of silver has caused anemia (Shouse and Whipple, 1931). It was suggested that long-term feeding of animals with silver salts may cause vascular hypertension, hypertrophy of the right ventricle, and thickening of the glomerular membranes (Olcott, 1950).

The chronic effects in man usually have taken the form of an unsightly permanent blue-gray discoloration of the skin, mucous membranes, and eyes known as "argyrosis" or "argyria." Although this is considered only a cosmetic defect, with no significant physiologic effect, some observers maintain that deposition in the kidney is associated with arteriosclerotic changes, and deposition in the eye, with poor night vision (Gettler, 1927; Velhagen, 1953).

Local and generalized argyria, rarely seen today, has been caused by medical use of silver by ingestion or injection. Topically applied silver ointments have been shown not to pass the dermal barrier; this ensures safety on contact with bathing water treated with silver preparations (Norgaard, 1954). Industrial poisoning is a more likely cause of argyria, which develops slowly after 2-25 years of exposure. Estimates from industrial exposure show that the gradual accumulation of 1-5 g of silver will lead to generalized argyria (Hill and Pillsbury, 1939). The exact quantities of silver stored are not known. A safe assumption would be that 50% of the intake is retained in the body. Thus, the interim drinking-water level of 50 µg/liter would be equivalent to a retention of 50 µg of silver per day and would result in an accumulation of 1 g in 55 years, to give a probable borderline argyria. However, the maximum measured silver concentration in drinking water was 30 µg/liter, which would mean 91 years to retain the quantity believed to produce argyria. The usual silver concentrations in public water supplies are even lower--about 2-3 µg/liter. Some states have more stringent standards, such as California (10 µg/liter) and Illinois (0.5 µg/liter). The Water Quality Criteria (1972) concluded that, "Because silver in waters is rarely detected at levels above 1 µg/liter, a limit is not recommended for public water supply source."

There is no evidence of any beneficial effect to be derived from the ingestion of silver in trace quantities.

Analysis. Silver ions in solution are unstable under many conditions, but the addition of ethylenediaminetetraacetic acid to collected natural-water samples has been found to be an adequate preservative (West *et al.*, 1967). Nitric acid is used by the U.S. EPA (1971) for stabilization.

With direct flame atomization, the detection limit for silver is 2 µg/liter. At the silver concentrations normally found in fresh water, some form of concentration is required for conventional atomization. An APDC-methylisobutylketone extraction at a pH of 2.8 is used by the U.S. Geological Survey (Brown *et al.*, 1970); similar procedures have been reported by others (Chao *et al.*, 1969; U.S. EPA, 1971; Traversy, 1971; Kinrade and Van Loon, 1974).

When a graphite furnace is used to increase sample atomization the silver detection limit is lowered to 0.005 µg/liter with direct sampling. Rattonetti (1974) determined silver in fresh water with flameless atomic absorption. The sampling boat and Delves cup methods offer detection limits of 0.2 and 1 µg/liter, respectively.

Conclusions and Recommendations. There seem to be no pressing research needs with regard to silver in drinking water. There seems to be little possibility that the addition of oligodynamic silver will have any place in public water supplies, and natural concentrations are so low that consideration should be given to taking silver off the list of substances included in primary drinking water standards.

Tin

Occurrence. Tin is seldom measured in natural water, in treated-water supplies, or at the tap. It is not indexed or mentioned in the NAS report on water-quality criteria in 1972 (EPA, 1973), nor was it included by Durum *et al.* (1971) in their reconnaissance of selected minor elements in U.S. surface waters. It is not listed in the *National Interim Primary Drinking Water Regulations* (EPA, 1975), nor in the U.S.S.R., European, or International drinking-water standards (Stoefen, 1973). Indeed, there are serious reservations about its valid determination, as noted below, and it is not included in *Standard Methods* (USEPA, 1976).

According to Beeson *et al.* (1976) public water supplies in 42 U.S. cities contained tin at 1.1-2.2 μg/liter, and water from 175 natural sources in west-central Arizona contained 0.8-30 μg/liter. Seawater contains 0.2-0.3 μg/liter. With emission spectrography, Durum and Haffty (1961) analyzed 59 samples of water from 15 rivers in the United States and Canada, of which 56 values were reported as zero, i.e., below the detection limit. The other three values were 1.3, 1.4, and 2.1 μg/liter.

Although tin is present in natural water only in traces, it may occur in industrial waste when water is stored for any length of time in tin-coated metal containers. Stannic and stannous chlorides are used as mordants for reviving colors and dyeing of fabrics, weighting of silk, and tinning of vessels. Stannic chromate is used in decorating porcelain. Stannic oxide is used in glassworks, in dye houses, and for fingernail polishes. Stannic sulfide is used in some lacquers and varnishes. Tin compounds are also used in fungicides, insecticides, and anthelmintics (McKee and Wolf, 1963). Finally, it should be noted that stannous fluoride is used in many toothpastes and consequently reaches municipal sewers. From various industrial processes and from municipal sewage, tin salts are bound to reach surface water or ground water; but, because many of the salts are insoluble in water, it is unlikely that much of the tin will remain in solution or suspension.

The major source of human intake of tin is canned foods and beverages. It is usually present in canned foods and drinks at levels less than 100 mg/kg, but much higher concentrations (greater than 1,000 mg/kg) may be present in some products after prolonged storage in closed nonlacquered cans or after some days of storage in open cans (Monier-Williams, 1949).

Schroeder *et al.* (1964) reported that natural foods, many from garden soils, contained tin ranging from zero to 0.5 mg/kg on a fresh-weight basis or up to 40 mg/kg in dry material. These values are considerably lower than those observed for canned food and beverages, but both sets are three or more orders of magnitude greater than the concentrations in water.

Very little information is available on airborne tin, but there may be danger to industrial and agricultural workers from the inhalation of atmospheric organic tin compounds, e.g., triphenyltin acetate, used in fungicides and insecticides (Pilmoor, 1968). Needless to say, the use of such pesticides increases the potential for intake of tin by ingestion of the pertinent crops as food. Dust sediments from industrial regions of Europe were reported by Morik and Morlin (1959) to contain tin at 10-15,000 mg/kg of dust.

Chemical Characteristics. In nature, tin is a decidedly minor trace component; it would not be expected to be found in natural water, except in very minor traces. Stannous hydroxide, $Sn(OH)_2$, is soluble in water at 25°C at about 1.6 ppm. At a pH considerably below that normally found in natural water, a much higher concentration may be possible; at a high pH, above the normal for natural water, tin may be part of an anion complex and dissolve in greater concentrations (USGS, 1959).

Metabolism. According to the FDA (U.S. FDA, 1975), "tin is poorly absorbed from the alimentary tract and most ingested tin is excreted via the feces. The tin that is absorbed is found mainly in the liver and lung with small traces in other tissues." In contrast, Kent and McCance (1941) found that at least half the dietary tin was excreted in urine. With a tin intake of 14.4 mg/day for 7 days, their subject excreted 7.2 mg/day in urine and 6.5 mg/day in feces. Hence, almost all the ingested tin was excreted.

It has been reported that the average human diet contains tin at 17.14 mg/day and that people can apparently tolerate 850-1000 mg/day (McKee and Wolf, 1963). In contrast, Schroeder et al. (1964) stated that the daily oral intake of tin by a man in the United States is between 1 and 30 mg/day, with typical intakes near 1-4 mg/day. Even the highest value (30 mg/day) is considerably lower than the 5-7 mg/kg of body weight at which toxic symptoms may appear (WHO, 1973). Tipton et al. (1969) reported that the daily intake of tin in the United States ranged from 0.10-100 mg, with an average of 5.0 mg. Again, such values are below those which might cause toxicity.

Higher concentration of tin are found in tissue from people in wealthier countries, probably as a result of greater use of canned foods (Schroeder et al., 1964).

Health Effects. There is no conclusive evidence that tin plays an essential biologic role in human nutrition (Browning, 1961). However, in rats maintained on purified amino-acid diets in trace elements, Schwarz *et al.* (1970) found that trimethyltinhydroxide, dibutyltinmaleate, stannic sulfate, and potassium stannate enhanced growth at tin dosages of 0.5-2.0 mg/kg of diet. Although the use of animal data to determine optimal intakes for people is subject to many possible errors, one might calculate that a 70-kg person would require tin at about 7.0 mg/day. Before tin can be conclusively considered as an essential trace element, effects should be demonstrated in several generations of various animals.

Inorganic tin is relatively nontoxic. DeGroot *et al.* (1973) fed inorganic tin compounds to rats for 13 w and found no toxic effects at tin concentrations of 450-650 mg/kg of diet. Indeed, some inorganic tin compounds had no effect on rats at three times that concentration.

Organic tin compounds, however, have demonstrated toxicities and have been used as fungicides, bactericides, insecticides, and anthelmintics (i.e., against intestinal worms). These compounds are generally of the type R_3SnX, where R is an alkyl group (especially ethyl or propyl) and X is an anion, such as chloride. The toxicities of organotin compounds have been reviewed by Barnes and Stoner (1959) and Poller (1970).

According to the FDA (U.S.FDA,), the "symptoms of acute tin toxicity" (to humans) "are nausea, abdominal cramping, diarrhea, and vomiting. These symptoms have often followed consumption of canned fruit juices containing 1,400 ppm tin, canned salmon containing 850 ppm tin, and vodka punch containing 2,000 ppm tin. The latter had been held in a tin can. Due to low intestinal absorption of tin, the acute toxic symptoms are probably due primarily to local irritation of the gastrointestinal tract.

"One hundred deaths in France resulted from capsules known as "Stalinon," used for treatment of staphylococcal skin infections. The capsules contained diethyltin-di-iodine (15 mg/capsule) and linoleic acid and were contaminated with mono-and triethyl tin." (U.S.FDA, 1975)

Analysis. Beeson *et al.* (1976) expressed serious reservations about the analytic determination of tin, especially at low concentrations. Many foreign substances in a sample interfere with the determination of tin by atomic absorption. Dry ashing is also subject to several errors, especially for most organotin compounds. As a result, many, or all, of the data reported on tin in water, food, and air can be accepted only with some reservations.

Direct flame atomization offers a tin detection limit of 10 μg/liter; however, tin can be determined to quite low concentrations by specialized atomization devices. With sodium borohydride as a reductant, Fernandez (1973) detected tin at 0.2 μg/liter in 20-ml sample. The acidity of the solution is critical and appears to be optimal near 0.2 N in hydrochloric acid. When the graphite furnace is used to increase sample atomization a detection limit of 0.1 μg/liter is possible (Everett *et al.*, 1974).

Conclusions and Recommendations. Inorganic tin is relatively nontoxic, but organotin compounds can be toxic at very high concentrations. Indeed, they are used as fungicides, insecticides, and anthelmintics.

Tin has seldom been determined in natural or municipally treated water. The few available data generally show concentrations about 1 or 2 μg/liter. In contrast, tin is present in most natural foods, and especially in canned products, up to 30 mg/day. This is three or more orders of magnitude higher than the probable amount in a liter of tap water.

The EPA has not set a limit for tin in its *National Interim Primary Drinking Water Regulations*. No limit is recommended or needed.

Perhaps the foremost research need with respect to tin is the development of a rapid accurate method of determination at the low concentrations expected in drinking water. Until such a method is available, reliable abundant data for natural or treated water cannot be expected.

Vanadium

The NAS has recently (1974) completed an extensive review on the medical and biologic effects of vanadium, which has been used in preparing this report.

Occurrence. A high vanadium concentration of 6.7 µg/liter has been reported in the Sacramento River, Sacramenta, California (Durum and Haffty, 1961). Kopp and Kroner (1967) observed detectable concentrations in 3.4% of the samples analyzed; the concentration ranged from 2-300 µg/liter, with an average of 40 µg/liter. One kind of pollution from vanadium must be noted when considering water. Residues from the milling and mining of vanadium are often heaped on the ground or used as landfills, thus being exposed to rainfall and ground water drainage, which could result in water pollution for many miles around. Industrial regulations now enforced are more likely to be sufficient to prevent this.

Chemical Characteristics. Vanadium does not occur naturally in highly concentrated forms. This is true despite the fact that it is as abundant in the earth's crust as zinc and nickel and occurs in at least 50 different mineral species. It usually occurs in some oxidized form usually as a metal vanadate. Vanadium can also be found in trace amounts in fossil fuels. The solubility in water of vanadium pentoxide and sodium metavanadate are 0.07 and 21.1 g/100 ml respectively. Vanadium can also form covalent bonds with organic molecules to yield organometallic compounds (NAS, 1974).

Metabolism. It has been reported that absorption of vanadium through the skin occurs from an approximately saturated (20%) solution of sodium metavanadate. Even with exposure to vanadium particles, the skin absorption appears to be of minor importance (NAS, 1974).

In an experimental study in which humans were exposed to vanadium oxide dust--with tests being run, before, during, and after exposure--the greatest amount of vanadium was found in the urine 3 days after exposure; none was detectable after a week. Fecal vanadium was at a maximum of 0.003 mg/g; none was detected after 2 w. All reactions to the exposure were respiratory. Coughing, mucus formation, rales, and expiratory wheezes were present, but did not last (NAS, 1974).

Vanadium concentrations in human tissues have been found to be less than 1 µg/g of ash, except in the lungs, where up to 108 mg/g (ashed material) has been reported after extremely high exposure. Vanadium pentoxide is readily absorbed from the lungs into the bloodstream (USEPA, 1975).

It would have to be concluded that absorption in the human body is extremely low; and it must be kept in mind that vanadium has not been proved to be essential to humans (Shakman, 1974).

Evidence seems to indicate that the excretion pathway of vanadium is through the kidney, regardless of the form administered. Rats, rabbits, and man all excrete sodium metavanadate via the urinary pathway. Man also excretes ammonium vanadyl tartrate in the urine (USEPA, 1975).

Health Aspects. A relatively large amount of vanadium (some 30,000 metric tons/year) enters the environment from man's activities, but no widespread detrimental effects have been identified. Presumably, man and other animals do not store or accumulate vanadium in hazardous amounts.

The degree of vanadium toxicity depends largely on the dispersion and solubility of vanadium aerosols in biologic media. Toxicity also depends on the valence; i.e., it increases with increasing valence, with pentavalent vanadium being most toxic. In addition, vanadium is toxic both as a cation and as an anion.

The oral LD_{50} of vanadium trioxide for albino mice is 130 mg/kg, while the LD_{50} for vanadium pentoxide and vanadium trichloride is 23 mg/kg (Roshchin *et al*., 1965).

The major signs and symptoms of acute vanadium toxicity in man are primarily respiratory. Aside from its acute inflammatory effect on the lungs, it appears to act mainly on various enzyme systems.

Chronic respiratory exposure to vanadium may decrease cholesterol synthesis, uncouple oxidative phosphorylation in liver mitochondria, and decrease urinary excretion of 5-hydroxyindoleacetic acid, with transient bilirubinemia and albuminuria.

Another symptom is the appearance of scattered allergy-like eczematous skin lesions. These are found, for the most part, on exposed skin. This allergic syndrome has been seen in workers and experimental animals. A persistent complication is a slight to moderate change in the mucous membranes of the upper respiratory tract, particularly the pharynx; but, no chronic bronchitis or changes in the lung have been reported. Permanent damage to the target organs, including the lungs, has never been conclusively established.

There is no evidence of any chronic oral toxicity (NAS, 1974).

Analysis. With a vanadium detection limit of 40 μg/liter, conventional flame atomization lacks sensitivity for direct determination in most samples. Crump-Wiesner and Purdy (1969) have studied various extraction systems and found that both vanadium (IV) and vanadium (V) are extracted from an aqueous solution at a pH of 3.8 with a cupferron-methylisobutyl ketone system. With direct sampling, the graphite furnace can be used to increase sample atomization with a detection limit of 5 μg/liter.

Conclusions and Recommendations. A limit of 0.1 mg/liter has been suggested in the U.S.S.R. as a maximum permissible limit for water basins (U.S. EPA, 1975).

The lack of data on acute or chronic oral toxicity is not surprising because of the extremely low absorption of vanadium from the gastrointestinal tract. Inhaled vanadium can produce adverse health effects, but the available evidence does not indicate that vanadium in drinking water is a problem.

Zinc

Zinc is considered an essential trace element in human and animal nutrition. This topic has been recently reviewed in Clinical Chemistry (1975) in a special issue on trace elements, by Sandstead (1974), by Underwood (1971), and in Toxicants Occurring Naturally in Foods (NAS, 1972). The recommended daily dietary allowances for zinc recently recommended by the NAS (1974) are as follows: adults, 15 mg/day; growing children over a year old, 10 mg/day; and additional supplements during pregnancy and lactation. As far as human health in the general population is concerned, the major concern is not with toxicity, but rather with marginal or deficient zinc intake. The available data indicate that the contribution of drinking water to the daily nutritional requirement for zinc is negligible under most circumstances.

Occurrence. In general, in streams and rivers, zinc is concentrated in sediments, but concentrations are quite low in running filtered water. It is reported that approximately 22,000 tons of zinc are used in fertilizers each year in the United States. The extent to which this may run off into rivers and streams is not known. Likewise, no significant body of data relative to the runoff into streams from dumps and metallurgic wastes has been found. Craun and McCabe's (1975) recent summary indicates that concentrations of zinc in finished water at the treatment plant are well below the 1962 drinking-water standard of 5 mg/liter. However, in areas of soft acidic water--in Seattle, Washington, and Boston, Massachusetts--pickup in the distribution system was noted in comparing water samples from the treatment plant with samples at the tap. In the Boston study, 35% of 108 samples showed pickup, but both mean and maximum concentrations in running water were well within the current limit (mean, 223 μg/liter; maximum, 1,625 μg/liter). In the more acidic water in Seattle, pickup of zinc was noted in 95% of samples, and 10% were in excess of the standard of 5 mg/liter. The maximum concentration found in the study was 5.46 mg/liter.

In rocks, zinc is most commonly present in the form of the sulfide sphalerite, which is the most important zinc ore. Zinc may replace iron or magnesium in certain minerals. It may be present in carbonate sediments. In the weathering process, soluble compounds of zinc are formed and the presence of at least traces of zinc in water probably is common. Concentrations of 40 ppm impart a strong astringent taste to water.

Food is the major source of zinc. This topic has been reviewed elsewhere (Sandstead, 1974; Underwood, 1971). Regarding industrial exposures, the acute metal fume fever generally attrubuted to zinc is a brief self-limited disease and is well known. In other types of industrial smelting operations, zinc, lead, and cadmium frequently occur together; the latter two are much more toxic than zinc. Where accidental discharge in water has been identified, it has been related to smelting and refining operations and has involved combined exposures to zinc, cadmium, and lead (Friberg *et al.*, 1974). Airborne zinc is generally not considered significant, as far as the general population is concerned.

Chemical Characteristics. Zinc chloride and sulfate are very soluble in water, but hydrolyze in solution and reduce the pH. If the pH is maintained by the presence of an excess of bicarbonates and other anions normally present in natural water, the solubility of zinc is likely to be controlled by the solubility of its carbonate and hydroxide. Zinc carbonate is soluble in pure water at 25°C to the extent of zinc at 107 mg/liter. The hydroxide is soluble only to the extent of zinc at 0.2 mg/liter. The pH at which zinc might precipitate as hydroxide is probably not reached in the presence of excess carbon dioxide in solution. At a very high pH, zinc may form anion complexes, but such conditions are not likely in natural water (USGS, 1959)

Metabolism. An earlier NAS committee (Sandstead, 1974) identified zinc balance data in humans as an area needing much more research. In general, animal studies indicate that, although zinc is distributed throughout the body, including bone, there is a small labile pool with a rather rapid turnover. The urinary excretion of zinc is generally less than 1 mg/day.

Health Effects. Diets grossly deficient in zinc have been found in Iran and Egypt. These have been associated with growth failure, loss of taste, and, in the postpubertal male, hypogonadism and decreased fertility. It is likely that factors in addition to zinc may also be involved. With the exceptions of diminution of taste discrimination and appetite, such conditions have not been identified in the United States, although there is a suspicion that some segments of the population are marginally zinc-deficient. Of interest is the recent finding that patients with sickle-cell disease may be zinc-deficient, owing at least in part to an increased loss of zinc in the urine.

Acute adverse effects of zinc include acute metal fume fever by the inhalation of fumes. There appears, on the basis of animal studies, to be a rather wide margin of safety between tap-water zinc content and oral doses that will produce toxicity (NAS, 1973). There have been reports of human cases of zinc poisoning associated with the prolonged consumption of water from galvanized pipes. In two adults, irritability, muscular stiffness and pain, loss of appetite, and nausea were reported when the water contained zinc in a concentration of 40 mg/liter, which is well above the current secondary drinking-water standard of 5 mg/liter.

There is no evidence that zinc in excess is carcinogenic, mutagenic, or teratogenic.

Zinc interacts with other trace metals (Sandstead, 1974, 1976). It clearly has a protective action against cadmium and lead. Animal data suggest that the zinc:copper ratio in the diet may be important. As noted above, there may be an interaction between zinc and iron. If these experimental observations are, in fact, important for human health, then it is possible that the ratio of zinc to these other metals in drinking water may be of some importance. This problem has not been explored.

Analysis. Most fresh-water analyses may be made by using atomic-absorption spectrophotometry with direct aspiration, with a zinc detection limit of 1 μg/liter. The EPA (1971) and the U.S. Geological Survey (Brown et al., 1970) procedures are typical. For low concentrations in fresh water, solvent extraction may be used. Mulford (1966), Paus (1971), Kinrade and Van Loon (1974), and Aldous et al. (1975) have found that the APDC-methylisobutylketone system extracts zinc at a pH of 2.8. Diethyldithiocarbamate (Joyner et al., 1967; Nix and Goodwin, 1970), dithizone (Sachdev and West, 1969), and dibenzyldithiocarbamate (Ichinose, 1974) are among other chelating agents used for concentrating zinc. The Delves cup and sampling boat (Paus, 1971) have been used for zinc analysis, with detection limits of 50 and 30 μg/liter, respectively. Fernandez and Manning (1971) and Surles et al. (1975) have demonstrated the use of the graphite furnace to increase sample atomization for fresh-water analysis, with a zinc detection limit of 0.001 μg/liter. Background correction appears to be essential in atomic-absorption spectrophotometry. In recent years, since the introduction of background correction, normal plasma zinc concentrations have consistently decreased.

Conclusions and Recommendations. Research needs have been proposed previously by an NAS Committee (Sandstead, 1974). These are related primarily to the zinc content of foods, the need to determine whether a significant proportion of American diets are either deficient or marginal in zinc, and whether specific segments of the population are genetically susceptible to zinc deficiency. The recommendations include:

1. Assessment of the availability of zinc in food to man.
2. Determination of human zinc requirements in relation to age and physiologic state.
3. Evaluation of the possible implications of the zinc:cadmium ratio for health.

4. Determination of the zinc status of various well-defined populations and relation of these findings to other measures of nutritional status.
5. Assessment of the effect of zinc supplementation and enrichment on the health status of well-defined populations.

In addition, animal data suggest that zinc is also protective against lead toxicity. This possibly significant interaction needs further investigation. As far as drinking water is concerned, the present drinking-water standard, assuming an adult water consumption of 2 liters/day, would permit the intake from drinking water of up to 10 mg/day, which is less than the estimated adult dietary requirement for zinc. The available data on drinking water, however, suggest that the amounts in drinking water are far lower than this. Another area requiring further investigation is related to the zinc content in the presence of soft water and the use of galvanized pipes. The present recommended primary interim standard of 5 mg/liter appears adequate for acceptable taste and appearance of drinking water.

Summary

Trace Metals. The Interim Primary Drinking Water Regulations list maximum allowable concentrations for six metallic elements - barium, cadmium, chromium, lead, mercury and silver. Ten additional metals were reviewed in this study - beryllium, cobalt, copper, magnesium, manganese, molybdenum, nickel, tin, vanadium and zinc. Sodium, which is also a metallic constituent, is considered in a separate section because the problems it poses are quite distinct from those associated with the other metallic substances.

Eight of these selected metals are known to be essential to human nutrition: chromium, cobalt, copper, magnesium, manganese, molybdenum, tin and zinc. Nickel and vanadium probably are essential also, and it is possible that barium can be beneficial under certain conditions. The toxic metals, lead, mercury and cadmium are believed not to be essential to humans. Beryllium and silver also are not known to be essential.

Elements that are beneficial in small quantities very often exhibit toxic properties when ingested in excessive amounts or concentrations. In assessment of the adverse health effects of such materials it is important not to overlook the deficiency problems that might be encountered if the substances were to be completely eliminated from water supplies.

Trace metals, usually in the form of ions, occur in water both as a result of natural processes and as a consequence of man's activities. Ground waters, because of long contact with rocks and mineralized soils, usually contain greater concentrations of trace metals than surface waters. There is considerable temporal and spatial variation in concentrations of trace metals in surface waters. Generally, the trace metal concentrations of rivers tend to increase from source to mouth and to vary inversely with discharge, which dilutes the natural and industrial contaminations.

Of the 16 metals studied the relative contribution of man's activities to the concentrations found in water supplies can be rated roughly as follows: very great-cadmium, chromium, copper, mercury, lead and zinc; high-silver, barium, molybdenum, tin; moderate-beryllium, cobalt, manganese, nickel and vanadium; low-magnesium.

Other important sources of trace metals in drinking water are water treatment processes and pickup of metallic ions during storage and distribution. Although a large fraction of the United States continues to receive water from ground sources or from impounded upland sources without treatment other than disinfection, most large surface supplies are subjected to treatment that includes coagulation, sedimentation, filtration and disinfection. Should trace metals occur in the raw water supply, these normal water treatment processes have either no effect or an uncertain one on removing the usual low level concentrations of these metals. Moreover, probable trace metal impurities in the technical-grade chemicals used may introduce additional concentrations into the treated water.

Control of the corrosive properties of the finished water is important to prevent increase in trace metal concentrations during storage and distribution. A wide variety of materials including several metals, alloys, cements, plastics and organic compounds are used in the pumps, pipes, fittings and reservoirs of distribution systems and home plumbing. Reactions, particularly of soft, low-pH waters, with materials of the distribution system very often have produced concentrations of iron, copper, zinc, lead and cadmium at the tap much greater than those in the raw or treated waters at the plant.

The positive correlations between "hard" water supplies and reduced cardiovascular disease is discussed in detail elsewhere in this report.

Adverse health effects associated with trace metals depend upon the total intake from all sources - water, air and food. As a general rule concentrations of trace metals in foodstuffs greatly exceed those found in drinking waters. Because the diet of most of the U. S. population is increasingly varied and comes from diverse geographical sources as a result of modern food distribution practices that counterbalance local excesses or deficiencies, the dietary intake of trace metals exhibits relatively small variation throughout the U. S. This factor is helpful in evaluation of maximum no-adverse-health-effect concentrations for drinking water.

Airborne exposure to trace metals is largely occupational through the inhalation of industrial dusts or fumes, except for lead, to which there is more general exposure from motor exhaust fumes. Most evidence for acute and chronic health effects is derived from data on occupational exposures; caution must be observed in extrapolation of these data to the general public.

All of the trace metals studied are known to exhibit toxic effects at some level of intake. Many of these, however, are at levels greater than the maximum concentrations found in drinking water. To include such materials in primary drinking water standards with a requirement for mandatory surveillance does not confer any health benefit. Adverse health effects from trace metals that are not found in excessive concentration in delivered water supplies can be avoided most readily by preventing the discharge of such contaminants into water in quantities that might increase concentrations to the maximum no-observed-adverse-health-effect level.

The following sections summarize the findings on individual trace metals.

Barium. It is rare to find barium in drinking water at a concentration in excess of 1 mg/liter because of the low solubility of barium sulfate. Natural and treated waters usually contain sufficient sulfate so that more than 1 to 1.5 mg/liter of barium cannot be maintained in solution.

Acid-soluble barium salts are very toxic, whereas insoluble compounds are benign. There has been no determination of the chronic effects of low levels of barium ingested over a long period of time. The chronic phase of poisoning is an occupational disability following prolonged exposure to barium dust.

It is recommended that animal studies involving long-term low-level ingestion of barium salts in water be carried out to determine possible health effects.

The Interim Primary Standard of 1 mg/liter for barium has been based on extrapolation from effects of industrial exposure to dusts of soluble barium salts. Insufficient data are available to estimate maximum no-adverse-health effect levels on the basis of water intake. The limit of 4 mg/liter of the U.S.S.R. is based on organoleptic factors. International and European Sandards of the World Health Organization do not list barium.

Beryllium. Beryllium is not likely to be found in natural waters greater than trace amounts because of the relative insolubility of beryllium oxides and hydroxides in the usual pH range of drinking waters. The sulfate and chloride are very soluble, but they hydrolyze quickly to the insoluble hydroxide.

Beryllium produces acute or chronic toxicity in animals when ingested continuously as beryllium sulfate in food or water only at levels in excess of 10 - 20 mg/kg of body weight per day or at concentrations greater than 5 mg/liter. Soluble beryllium has been shown to be transported in the bloodstream to bone where it has been found to induce bone cancer in animals, but the data are insufficient to allow estimation of risk.

Prolonged inhalation of dusts containing beryllium is known to produce pulmonary findings similar to sarcoidosis. However, increased incidence of lung cancer among workers exposed to beryllium-containing dusts has not been found.

No maximal allowable concentration for beryllium has been listed in the Interim Primary Drinking Water Regulations, nor has the WHO recommended a maximum limit. The U.S.S.R., however, has set a limit of 0.2 μg/liter. Until now the maximum concentration of beryllium found in U. S. surface waters has been 1.2 μg/liter and in finished U. S. drinking waters has been 0.17 μg/liter. Only 1.7% of drinking water supplies examined have been found to contain any detectable beryllium.

Additional studies of the frequency of occurrence and concentration levels of beryllium in natural waters are needed to determine the extent to which it presents a hazard to health.

Cadmium. Cadmium is not known to be an essential or beneficial element. It has been found in 2-3% of U. S. surface waters, generally in concentrations not exceeding a few milligrams per liter due to the low solubilities of cadmium carbonate and hydroxide at pH greater than 6. Only 0.2% of the supplies in the Community Water Supply Survey showed cadmium in excess of 0.01 mg/liter. In addition to its geological sources, cadmium enters water from the discharge of plating wastes and by the action of corrosive waters on distribution piping and home plumbing.

Total daily intake from air, water, food and tobacco ranges from 40 μg/day μg/day for the rural nonsmoker on a low cadmium diet to 190 μg/day for the urban smoker on a high cadmium diet. Drinking water contributes only a small fraction (<5%) to this total intake.

Chronic ingestion of cadmium at levels greater than 600 μg/day in combination with several other necessary predisposing factors was found to be responsible for the onset of "Itai-Itai" disease in Japan. Dietary intake of amounts in excess of a milligram per day is needed for appearance of acute toxicity. Major toxic effects are on the kidney; data indicate that the toxicity of cadmium is related to the zinc-cadmium ratio within the organs. Both zinc and calcium may be protective against cadmium toxicity. Persons deficient in these elements, and especially lactose-intolerant persons who are likely to be calcium-deficient, may constitute a high risk group toward cadmium. There have been some indications of carcinogenic and teratogenic effects of cadmium in animal studies, but dose-response relationships are unknown. Cadmium has also been implicated as a factor in hypertension.

Insufficient data are available for establishment of a maximum no-observed-adverse-health-effect value. It may be noted, however, that at a concentration level of 10 µg/liter in water, cadmium contributes only about 20% of the normal total daily adult intake with water consumption at two liters/day. Both the WHO and the U.S.S.R. have set the maximum allowable limit for cadmium at 10 µg/liter.

Chromium. Microgram amounts of chromium, derived primarily from food, are essential for maintenance of normal glucose metabolism. But chromium (VI) is known to be toxic, principally on the basis of information from respiratory occupational exposures. Increased risk of lung cancer among those exposed occupationally to chromium (VI) has been established.

Although inhaled hexavalent chromium may cause cancer of the respiratory tract, the IARC working group concluded "there is no evidence that non-occupational exposure to chromium constitutes a cancer hazard."

Concentrations of chromium found in natural waters are limited by the low solubility of chromium (III) oxides. A study of more than 1,500 surface waters showed a maximum chromium content of 0.11 mg/liter, with a mean of 0.01 mg/liter.

Little information is available on average total daily intake of chromium in the U. S., although it appears to be in the range of 60 - 280 µg/day. It has been suggested that diets containing mostly processed foods may be chromium-deficient. Tissue chromium in U. S. adults has been shown to decline with age.

In addition to the beneficial effect of chromium on glucose metabolism, there have been indications from animal studies that chromium deficiency may induce atherosclerosis.

Toxicity of chromium depends on the valence. No toxic effects were observed in rats when drinking water contained 25 mg/liter of trivalent chromium for a year or 5 mg/liter for life. Acutely toxic doses of trivalent chromium fall in the range of grams per kilogram of body weight. Hexavalent chromium was also tolerated at the 25 mg/liter level for a year by rats. Dogs showed no effects with 11 mg/liter over a 4 y period. Higher doses are toxic, however, producing erosion of the gastrointestinal tract and kidney lesions.

The maximum limit of the Interim Primary Drinking Water Standards, 0.05 mg/liter, is only one-hundredth of the maximum no-observed-adverse-health effect concentration. The European Standards of the WHO and Japanese Standards give the same acceptable limit, but set it in terms of hexavalent chromium only. The U.S.S.R. has limits of 0.1 mg/liter chromium (VI) and 0.5 mg/liter total chromium, based on organoleptic factors.

More information is needed on the carcinogenic potential of ingested chromium (VI) and chromium (III). If it becomes clear that highly toxic or carcinogenic effects occur only with chromium (VI) and a suitably sensitive analytical technique is available, then the standard might be set for chromium (VI) alone. In view of the U. S. trend toward dietary chromium deficiency and the suggestion that chromium protects against atherosclerosis, it seems advisable to investigate whether greater allowed concentrations are without adverse health effects, as some animal experiments suggest.

Cobalt. Cobalt is an essential element as a component of vitamin B_{12}. This is its only known nutritional function. Excessive intake of cobalt may be toxic with the most notable instance being the association of congestive heart failure with the consumption of beer containing about 1.5 mg/liter of cobalt.

Cobalt has been observed in natural waters only in trace amounts. Most waters have no detectable cobalt and values greater than 10 μg/liter are rare. The maximum recorded value in any of several broad studies was 99 μg/liter.

The major source of cobalt is food; concentration in green, leafy vegetables may be as great as 0.5 mg/kg dry weight. Normally, less than 1% of total intake of cobalt is derived from aqueous sources.

Acute toxic effects in animals have been observed only with daily cobalt doses greater than several mg/kg of body weight. Chronic cobalt toxicity has been observed in children taking cobalt preparations to correct anemia at daily doses of 1 - 6 mg/kg body weight.

The Interim Primary Drinking Water Standards do not list cobalt, nor has the WHO recommended a limit in its International or European standards. The U.S.S.R. has set a limit of 1.0 mg/liter.

Because the maximum no-observed-adverse-health-effect level is more than an order of magnitude greater than the concentration found in any natural water or drinking water supply, there appears to be no reason at present to regulate the concentration of cobalt in drinking water.

Copper. Copper is an essential element for both plants and animals; it is a component of several enzymes that perform important physiological functions.

Copper is a minor constituent of natural waters, the concentration ranging from 1-280 μg/liter in a survey of 1600 surface waters of the United States. Concentrations may be increased in drinking waters to several mg/liter by corrosion of copper piping in distribution systems, particularly with soft, non-alkaline waters. Copper may also be released into water in industrial discharges and has been used for algal control in reservoirs at concentrations of a few tenths of a milligram per liter.

Average total intake of copper is about 2.5 mg/day, so that when water contains more than 1 mg/liter of copper, the intake from water may equal or exceed that from food.

The general health hazard from excess copper intake at a level of a few milligrams per liter appears to be small, but few people are adversely affected by ingestion of even trace amounts of copper. This disorder of copper metabolism, called Wilson's disease, can be arrested by the use of chelating agents. Individuals with deficiency of glucose-6-phosphate dehydrogenase may be sensitive to copper.

The U.S.P.H.S. Drinking Water Standards (1962) recommended a limit for copper of 1 mg/liter based on organoleptic rather than health effects. Because no general adverse-health-effects have been observed at the organoleptic limit and because the few individuals with metabolic deficiency are at the mercy of total copper intake rather than copper in water, there is no hygienic reason to impose a limit lower than the presently accepted secondary standard.

Lead. No beneficial effects of lead on human or animal development have yet been found. Although acute lead poisoning is rare, chronic lead toxicity is severe and occurs even with low daily intake of lead (< 1 mg) because of its accumulation in bone and tissue.

The natural lead content of surface waters is generally small. In a survey of nearly 1,600 raw surface waters 20% were found to contain detectable concentrations of lead and these had a mean concentration equal to 0.023 mg/liter. The lead concentration in municipal supplies at the tap may be much greater, however, for soft, low pH (agressive) waters will dissolve lead from service connections, lead-lined household piping or soldered joints. Lead concentrations in excess of the interim standard of 0.05 mg/liter were found in 1.4% of the water systems tested in the Community Water Supply Survey. The maximum value was 0.64 mg/liter.

The mean concentration of lead in U. S. drinking waters has been estimated to be 0.013 mg/liter. Consumption of 2 liters/day per capita gives a mean daily intake of 26 μg.

Lead intake from food varies greatly with mean daily values estimated at 100-300 μg per capita for adults. Average water intake is considerably less than that from food, but when the concentration in water is close to or exceeds the interim standard of 0.05 mg/liter, water intake approaches that from food.

Absorption of lead from dietary sources, either food or water, is estimated to be about 10% for adults. Daily lead absorption from food is, then, 10-30 μg, while absorption from water ranges from an average of 3 μg to 10 μg or more, when water having a lead concentration of 0.05 mg/liter or greater is ingested at 2 liters/day.

The daily intake from air also ranges widely, and is greatest among city dwellers, situations. For a daily inspiration volume of 20 m³ for adults and a lead concentration of 3 μg/m³ in urban air, the per capita daily intake is 60 μg. The absorption percentage from air is about 40%, however, so that the daily quantity absorbed is 24 μg, a value comparable with the dietary absorption.

The sum of the estimated absorptions from the various routes, 30 - 60 μg/day, is already at the maximum no-observed-adverse-health-effect values of 50 - 60 μg/day.

Children, and especially inner-city urban children, are a special risk group with regard to lead toxicity. A primary reason is that absorption of lead from food and water is 40-50% for 2-3 year old children, rather than the 5 to 10% characteristic of adults. Also, water intake per kilogram of body weight is considerably greater for young children than for adults. Moreover, lead concentrations in urban air increase with proximity to the ground, so that urban children tend to have increased intake from this source. Young children also have the added risk of ingestion of flaking lead-based paints especially in depressed, older, urban areas.

Dietary lead intake for a 2-y old child (12 kg) has been estimated to be 100 μg/day (8.3 μg/kg/day); with water at the present 0.05 mg/liter limit and a consumption of 1.4 liter/day, and with air intake about 10 μg/day, the estimated total intake for a 2 y old would be close to 190 μg/day, not including other possible sources. If the water contains 0.1 mg/liter of lead, the present allowable limits of the WHO European standards and of the U.S.S.R., then an intake of nearly 260 μg lead/day for a 2 y old child can be estimated. With this intake an overall absorption close to 100 μg/day can be estimated, a value suggesting that the allowable concentrations of the WHO European standards and the U.S.S.R. may fail to provide adequate protection for children.

Major chronic adverse effects of lead are produced in the hematopoietic system, central and peripheral nervous systems and kidneys. Disturbance in heme synthesis is considered to be the most sensitive effect. There is a detectable increase in red-cell protoporphyrin in women and children with blood lead concentrations greater than about 25-30 μg/dl. For men occupationally exposed, the maximum no-observed-adverse-health effect level appears to be somewhat greater at 50-60 μg/dl.

Results of studies in the Boston area indicate that increased blood levels of lead will occur in children when the water supply contains 0.05 - 0.1 mg/liter of lead. Thus, the interim limit of 0.05 mg/liter may not provide the margin of safety to safeguard the high risk population in urban areas. Although satisfactory for a 70 kg adult, the WHO recommendation of 5 μg of lead per kg/day as a safe total daily intake cannot be met for a 12 kg child when the water supply contains as much as 0.05 mg/liter. It is concluded that the no-observed-adverse-health-effect level cannot be set with assurance at any value greater than .025 mg/liter.

Manganese. Manganese resembles iron in its chemical behavior and occurrence in natural waters, but is found less frequently and usually at lower concentrations than iron. Manganese, like iron, is an essential trace nutrient for plants and animals. It is not known whether human manganese deficiency occurs in the United States. The solubility of the several oxidation states of manganese (II, III, and IV) depends upon the pH, dissolved oxygen and presence of complexing agents. Occasionally, deep lakes or impounding reservoirs that contain organic sediments under anerobic reducing conditions can distribute several mg/liter of Mn^{+2} throughout the water body during "turnover" mixing. Normally, however, manganese in natural surface waters is less than 20 μg/liter.

Manganese can be absorbed by inhalation, ingestion, and through the skin; the consequences of this have been recently reviewed in depth by the National Academy of Sciences. It has been known that the occupational inhalation of manganese dusts results in a disease of the central nervous system resembling Parkinsonism and a form of pneumonia.

Ingestion of manganese in moderate excess of the normal dietary level of 3-7 mg/day is not considered harmful. A reported outbreak of manganism in Japan was attributed to drinking well water containing about 14 mg/liter of manganese.

The maximum concentration of manganese found in the 1975 Survey of Interstate Water Supply Systems was 0.4 mg/liter except for samples from two Alaskan airports which showed 1.0 and 1.1 mg/liter. A total of 669 supplies were examined. Similarly the maximum concentration found in the 1969 Community Water Supply Survey was 1.1 mg/liter from 969 supplies. Both these maximum concentrations are an order of magnitude less than minimum concentrations at which adverse health effects are observed. Moreover, even with manganese at 0.5 mg/liter the intake of manganese from water would be only about 15% of the normal total dietary intake of manganese.

Because concentrations of manganese found in water supplies are much less than those at which adverse health effects have been observed and because the regulation of manganese for esthetic and economic reasons is also far more stringent than would be required for reasons of health, there seems little need to establish a maximum no-observed-adverse-health-effect value.

Magnesium. Magnesium is an essential element in human, animal, and plant nutrition. It is geologically ubiquitous and the industrial uses of its salts are legion. The average U. S. adult ingests between 240 and 480 mg of magnesium per day. Magnesium intake from 3.6 - 4.2 mg/kg of body weight are believed to be adequate to maintain magnesium balance, which is closely regulated by normal kidneys. The median concentration of magnesium in the water of the 100 largest U. S. cities was reported at 6.25 mg/liter with a maximum of 120 mg/liter. It can be higher, especially in arid western states.

An excess of magnesium in the diet is seldom harmful, for it is generally excreted promptly in feces. High concentrations of magnesium sulfate in drinking water have a cathartic effect on new users, but a tolerance is soon acquired. Excessive magnesium in body tissues and extracellular fluids occurs only as a result of severe kidney malfunction. Magnesium deficiency in humans may occur in alcoholics, persons performing hard labor in hot climates (because magnesium is excreted in perspiration), those with certain endrocrine disturbances, and patients using potent diuretics. Such deficiencies can best be overcome by oral administration of magnesium compounds.

The National Interim Primary Drinking Water Regulations do not contain a limit for magnesium, nor did the 1962 USPHS drinking water standards. The U.S.S.R. has set no limit, but the World Health Organization has recommended a maximum of 150 mg/liter. In view of the fact that concentrations of magnesium in drinking water less than those that impart astringent taste pose no health problem and are more likely to be beneficial, no limitation for reasons of health appears needed.

Mercury. Mercury is a comparatively rare element. It is relatively insoluble in the inorganic form and can exist only in extremely small quantities under natural conditions. Recent measurements show that only 4% of water supplies contain mercury at concentrations greater than 1.0 µg/liter. Industrial use has resulted in increased environmental contamination. The health effects of populations occupationally exposed to mercury and mercuy compounds has long been recognized, but but the problem of contamination of the general environment is of recent origen.

Inorganic mercury in bottom sediments can be transformed biochemically to injurious methylmercury or other organic mercurial compounds. The organic form readily enters the food chain with concentration factors as great as 3000 in fish.

Several investigators have estimated the blood levels of mercury at which the identifiable symptoms of mercury intoxication occur. These levels may be obtained with a steady mercury intake of from 4-14 µg/kg/day. This would be 240-840 µg/day for adults and 80-280 µg/day for children.
It is estimated that the normal diet will contribute about 10 µg/day of mercury. With daily intake of 10 µg from food and 4 µg from water it appears that there is considerable margin of safety. However, those individuals regularly consuming fish from contaminated areas may exceed the normal intake by a factor of three or more and thus constitute a high risk population.

There is no indication that concentrations of mercury in drinking water or air have contributed in any significant way to methylmercury intoxication of the general population. The interim standard limits the daily intake to 3 - 4 µg/day. Nearly all public water supplies in the U.S. contain less than 2 µg/liter of mercury. The WHO has set no limit and the U.S.S.R. has a maximum permissible concentration of 5 µg/liter.

Molybdenum. Soluble molybdate ions are present in trace concentrations in many surface waters, primarily as a result of discharge of industrial wastes but also as a product of natural weathering of molybdenum-bearing soils. Both suspended insoluble molybdenum disulfide and soluble molybdates are present in streams draining areas where molybdenum ore is mined and processed, especially in Colorado and New Mexico.

Typical diets contain on the order of 100-1,000 µg/kg, whereas typical surface waters (except those draining mining areas) contain less than 100 µg/liter, with median values about

10 µg/liter. Hence, water is a minor factor in the total molybdenum intake in most locations. Since some finished waters have been reported to contain as much as 1.0 mg/liter, some water intake may provide as much as 2,000 µg/day of molybdenum. More information is needed about adverse effects of molybdenum at these levels to deal properly with such supplies.

Molybdenum poisoning has rarely been observed in humans. Although it has been implicated for gout in Armenia and for a bone-crippling disease in India, more information is needed to establish cause-and-effect relationships.

Molybdenosis in livestock is a significant toxicological problem in many areas of the world. Consumption of molybdenum-rich forage by cattle and sheep causes severe diarrhea (scouring) that sometimes results in death. It can be prevented or ameliorated by the administration of copper, but the relationship of molybdenum, copper, and sulfate-forming compounds need further study in animal metabolism.

The U.S.S.R. has established a limit for molybdenum of 0.5 mg/liter in open waters, but the World Health Organization has not promulgated a limit.

Nickel. Nickel may occur in water from trace amounts of a few micrograms per liter to a maximum of 100 µg/liter. At these levels the daily intake of nickel from water ranges from less than 10 µg/day to a maximum of 200 µg/day as compared to a normal food intake of 300-600 µg/day. Available information indicates that nickel does not pose a toxicity problem because the absorption from food or water is low. The principle reason for considering nickel stems from epidemiological evidence that occupational exposure to nickel compounds through the respiratory tract increases the risk of lung cancer and nasal-cavity cancer. There is difficulty in separating the effect of nickel from the simultaneous inhalation of other carcinogens including arsenic and chromium.

Because of the generally low concentration of nickel in drinking water and its reported low oral toxicity, there is no present need to set primary health effect limits for nickel in water. WHO and the U.S.S.R. have set no standards for nickel in drinking water.

Silver. Trace amounts of silver are found in some natural waters and in a few community water supplies. It has not been detected at levels exceeding the interim standard of 50 µg/liter. Colloidal siver consumed in large doses - several hundred mg/kg of body weight can cause anemia and possibly death. The main chronic effect in man is "argyria". Argyria is a cosmetic defect once caused through medical or occupational exposure to silver preparations. It is rarely encountered now. Dosages of from 1 - 5 g of silver are sufficient to produce this syndrome.

On the assumption of 50% absorption of silver, consumption of 2 liters/day of water containing 0.005 mg/liter of silver would result in an accumulation of 1 g of silver over 55 years.

Since silver ion has not been detected in water supplies in concentrations greater than half the no-observed-adverse-health-effect level, regulation of its concentration as a primary standard would appear to be unnecessary.

Tin. There is some indication that tin may be a beneficial micronutrient, although it has not been conclusively demonstrated that tin is an essential trace element in human nutrition. Inorganic tin is relatively non-toxic, but organotin compounds can be toxic at high concentrations. Indeed, they are used as fungicides, insecticides, and antihelminthics.

Tin has seldom been determined in natural or municipally treated water. The few available data generally show concentrations of the order of one or two µg/liter. In contrast, tin is present in most natural foods, and especially in canned products, to the extent that the normal human ingestion varies from 1.0-30 mg/day which is three or more orders of magnitude higher than the probable concentration in a liter of tap water.

EPA has not set a limit for tin in its National Interim Primary Drinking Water Regulations. In view of the foregoing considerations, no regulation seems necessary.

Vanadium. Vanadium is a trace metal which has been introduced into the environment in large quantities. Fresh surface waters show concentrations in the 2-300 µg/liter range, but with low frequency of detection. The data are limited on levels in finished drinking waters, but vanadium concentrations up to 19 µg/liter have been reported.

Occupational exposure to pentoxides and trioxides of vanadium leads to ear, nose and throat irritation and generally impaired health. The consequences of exposure to vanadium in air, water and food have been reviewed recently. There is no evidence of chronic oral toxicity.

Vanadium is considered a beneficial nutrient at µg/liter levels, having been suggested as protective against atherosclerosis.

Zinc. Concentrations of zinc in surface water are correlated with man's activities and with urban and industrial runoff. The solubility of zinc is variable, depending upon the pH of the water. In the same manner as lead and cadmium, zinc is dissolved in concentrations as great as several mg/liter from galvanized pipes and tanks in soft water systems. Concentrations ranging from 2 - 1200 µg/liter were detected in 77% of 1577 surface water samples and 3 - 2000 µg/liter in 380 drinking waters.

Zinc is relatively nontoxic and is an essential trace element with recommended minimum intake levels of 15 mg/day for adults and 10 mg/day for children over one year of age. A wide margin of safety exists between normal intake from the diet and those likely to cause oral toxicity. Concentrations of 30 mg/liter or more impart a strong astringent taste and milky appearance to water. Some acute adverse effects have been reported from consumption of water containing zinc at 40 - 50 µg/liter. There are no known chronic adverse effects of low level zinc intake in diet, but human zinc deficiency has been identified.

The presently established standard (USPHS) for zinc in drinking water is a "recommended" or secondary standard based on the threshold of the metallic taste at about 5 mg/liter. The WHO recommends the same limit; however, the U.S.S.R. has established a limit for zinc at 1.0 mg/liter for other than health reasons.

Other Inorganic Constituents

Arsenic

Occurrence. Arsenic is widely distributed in low concentrations in the waters of the United States. (Durum, 1974). In one study of selected minor elements in 720 samples of U.S. surface waters, the concentration of arsenic ranged from less than 10-1,100 micrograms/liter (10-1,100 ppb). A study by the U.S. Geological Survey (USGS, 1970) of river waters revealed that the median concentration of arsenic was less than 10 μg/liter, the lower limit of detection, but 22% of the samples had concentrations of 10-20 μg/liter (Table V-12). In this survey, except for local anomalies where arsenic concentrations could be traced to urban waters or to industrial sources, no major regional differences could be detected in average values or in percentage of contaminated samples.

The distribution of arsenic in waters and sediments of the Puget Sound region (Washington) has been studied by Crecelius and Carpenter (1974). A large copper smelter in the area releases about 100 tons of arsenic per year into the atmosphere in stack dust and about the same amount in liquid effluent directly into Puget Sound.

Arsenic

TABLE V-12
Regional Summary Of Arsenic in U.S. Surface Waters

Region	Maximum, μg/liter	Minimum, μg/liter	Median, μg/liter	Proportion <10μg/liter, %	Proportion >10μg/liter, %
New England and Northeast	60	<10	<10	80	20
Southeast	1,110	<10	<10	70	30
Central	160	<10	<10	75	25
Southwest	10	<10	<10	87	13
Northwest	30	<10	<10	86	14

From Durum *et al.*, 1971.

The concentrations of arsenic in Puget Sound waters are 1.5-2.0 mg/liter, except for surface waters within a few miles of the smelter, where they may reach 1,000 mg/liter. Away from the immediate smelter area, the concentrations of arsenic in the Sound are not likely to rise above 1-2 mg/liter because of the 1/2-y replacement time for waters in Puget Sound.

There have been a number of reports of isolated instances of higher than usual concentrations of arsenic in well waters. Lassen County, California (Goldsmith et al., 1972), was one such area. It was examined because of arsenic in well water ranging from 100 mg/liter or less to 1,400 mg/liter--well above maximal allowable standards. This compares with the 10 µg/liter recommended by the U.S. Public Health Service and the 200 mg/liter maximum in International and European drinking-water standards. The Lassen County study indicated that when the arsenic concentration in water was above 50 mg/liter, storage in hair increased, but there was no evidence of specific illness associated with concentrations up to 1,400 mg/liter.

In Perham, Minnesota, a newly bored well was associated with illness in 13 people whose hair samples contained arsenic at 17-1,680 micrograms/gram. The well water serving these patients contained arsenic from 11,800-21,000 mg/liter; this was later determined to come from ground contamination by residual arsenical grasshopper bait (Feinglass, 1973).

Antofagasta, a city of 130,000 in Chile, had a water supply containing high quantities of arsenic (800 ppb) between 1958 and 1970. The source of the high arsenic content was the Toconce River, whose waters come from the Andes Mountains at an altitude of 3,000 and were brought 300 km to Antofagasta (Borgono and Greiber, 1972). At the begining of the 1960's, the first cutaneous manifestations were noted, in children. There were several severe cases, including a few fatal ones, of arsenism at the Calvo Mackenna Hospital in Santiago. The principal finding was the close relation between the prevalence of cutaneous lesions (over 30% of the population) and the exposure to drinking water with a high arsenic content. The arsenic content of the hair and water supplies decreased markedly after action was initiated to clean up the water supply by opening a new water-treatment plant.

Natural sources, including the erosion of surface rocks, probably account for a significant portion of arsenic found in surface water and groundwater. Fleischer (1973) noted that fumarolic gases associated with volcanism have been reported to contain arsenic at up to 700 ppb, and waters of hot springs contain up to 13,700 ppb. Otherwise, scattered data for arsenic in groundwater indicate low concentrations, often below the limit of detection and perhaps averaging around 1 µg/liter (ppb). Isolated instances of arsenic in such concentrations as to warrant surveillance have been reported.

Arsenic is found in many foods; it occurs naturally in some and is introduced into others by way of feeds and pesticides. Crustaceans and other shellfish may contain up to 170 ppm (Frost, 1967). Apples that have been sprayed with lead arsenate to control codling moths might contain as much as 2 mg of residue. Wine and cider may contain arsenic, but it is usually removed during processing. Wine yeasts have been shown to contain arsenic in amounts up to 180 ppm, and baker's yeast up to 17 ppm. Meat may contain traces of arsanilic acid that has been used as a growth additive in cattle and poultry feeds. Theoretically, these additives are discontinued several days before marketing, and, in fact, the FDA allows an animal tissue arsenic content of 2.65 µg/g. There has been considerable speculation about the addition of any arsenic to the diet of animals. Arsenic was found in 3.2% of samples of food items examined in the United States during a marketbasket survey; residues ranged between 0.1 and 4.7 µg/kg (Cummings, 1966). The daily intake in the United States is calculated to be 0.137-0.330 mg (Duggan and Lipscomb, 1969).

Arsenic occurs in the earth's crust in concentrations averaging 2.0 ppm (Fleischer, 1973). It is concentrated in shales, clays, phosphorites, coals, sedimentary iron ore, and manganese ores. In the United States, arsenic is produced (and distributed into the environment) largely as a result of smelting nonferrous-metal ores, particularly copper. Recent analyses of petroleum show a median arsenic concentration of 90 ppb, but there are few data. Superphosphate fertilizer made by treatment of phosphorite with sulfuric acid has been reported to contain as much as 0.1% arsenic.

Metabolism. The metabolism of arsenicals by mammalian systems has been reviewed in detail by Frost (1967), Lisella et al. (1972), Harvey (1975), and others. The present discussion summarizes the available information.

Absorption. Water-soluble arsenicals are readily absorbed through the gastrointestinal tract, lungs, and skin; some nonpolar organic forms are also absorbed from the intestine and skin (Hwang and Schanker, 1973; Tarrant and Allard, 1972). Arsenic trioxide is only slightly soluble in water and is not well absorbed. Pentavalent arsenic, As (+V), whether inorganic or organic, is better absorbed than the trivalent form, because As (+V) is less reactive with membranes of the gastrointestinal tract. Arsenites are generally better absorbed through skin than arsenates, and absorption depends heavily on lipid solubility of the compound.

Some of the selective toxicity of arsenic is explained by arsenates penetration of insect cuticle more rapidly than of human skin. Arsenite is more toxic to humans than arsenate and is more readily absorbed through human skin (Harvey, 1975).

Distribution. Arsenic is distributed primarily to the liver, kidneys, intestinal wall, spleen and lungs. The extent to which arsenic is taken up by these tissues depends on the rate of exposure and the chemical form.

In guinea pigs, rabbits, apes, and humans, radioarsenite (^{74}As) injected subcutaneously was distributed to muscles and other tissues (Hunter et al., 1942). Sodium arsenite (^{74}As), that was injected intramuscularly to rabbits was found mainly in liver, kidneys, and lungs (Ducoff et al., 1948).

Arsenic is immobilized by binding to sulfhydryl groups in the keratin of hair and nails. Deposition begins within 2 w after administration, and the arsenic deposited may remain for the lifetime of the hair or nail. In this way deposition also serves as an excretory mechanism.

Excretion. There is a great deal of confusion in the literature regarding accumulation of arsenic. Rats (and possibly cats) appear unique, for they accumulate arsenic in the blood, bound to hemoglobin, whereas in other species there is no accumulation (Lanz et al., 1950; Hove et al., 1938; Peoples, 1964). Arsenate is rapidly excreted in the urine (Lanz et al., 1950; Ginsburg, 1965). Arsenate, however, can be reabsorbed by the proximal tubule in the dog kidney and re-excreted as arsenite (Ginsburg, 1965). Arsenite is excreted slowly in the urine, and it can take up to 10 days to completely excrete a single dose of parenterally administered trivalent arsenic (Hunter et al., 1942). It appears that arsenite is slowly oxidized to arsenate in the body and filtered into the urine in the pentavalent form.

Mealy et al (1959) suggested that arsenite is excreted by humans in three phases. More than 95% of radioactive sodium arsenite (^{74}As) injected intravenously into five human volunteers was excreted in the first 11 h after administration, most of the balance was excreted at a constant low rate over the next 155 h, and the remainder was excreted at an even lower rate. An alternative explanation for this observation is that the three phases are artifacts of the sampling time.

Administration of arsenic to cows does not appear to influence arsenic concentrations in milk, although tissue concentrations increased (Peoples, 1964). Arsenic acid (Peoples, 1964), lead arsenate (Marshall *et al.*, 1963), and arsenic trioxide (Fitch *et al.*, 1939) given to cows are readily excreted as pentavalent arsenic in the urine.

Transformation. Little is known about the biotransformation of arsenic in man in spite of the long use of arsenicals as pharmaceuticals and pesticides.

Arsenic inhibits the activity of many enzymes by reacting with sulfhydryl groups. In most cases the arsenical is converted to the trivalent form as an arsenite or arsenoxide (R-As=O). This active form of arsenic then combines with two sulfhydryl groups (often from two protein molecules) to form such products as R-As-(S-protein)$_2$ (Harvey, 1975). Intramolecular reactions also occur when arsenic combines with both sulfhydryl groups of α-lipoic (thioctic) acid to form a six-membered ring compound. Such reactions are considered responsible for much of the toxic action of arsenicals.

Health Effects. The toxicity of various arsenic compounds is extremely variable and depends on the species exposed, the formulation of the arsenical, the route of exposure, and the rate and duration of exposure. An assumption that all arsenic compounds are equally toxic is wrong and dangerous. Although man and other animals are susceptible to arsenic poisoning, there is a wide variation among species in susceptibility to a specific arsenic compound. Similarly, there is a wide variation in toxicity of the various arsenical formulations to a given species. Because of the many factors influencing the toxicity of arsenic, there is little point in attempting to state its toxicity in terms of milligrams per kilogram of body weight. It may be said, however, that the lethal oral dose of the more toxic arsenic compounds in most species appears to be 1-25 mg/kg of body weight, whereas the lethal dose for the less toxic compounds may range from 10-400 times this amount (Buck *et al.*, 1973; Penrose, 1974).

Groups of arsenic compounds can be listed as follows in decreasing order of toxicity (Penrose, 1974):

Arsines (trivalent, inorganic or organic)
Arsenite (inorganic)
Arsenoxides (trivalent with two bonds to oxygen)
Arsenate (inorganic)
Pentavalent arsenicals, such as arsonic acids
Arsonium compounds (four organic groups with a positive charge on arsenic)

Metallic arsenic

Arsine gas is an indirect hemolytic agent due to its inhibition of red-cell catalase, which leads to accumulation of hydrogen peroxide which in turn destroys the red-cell membrane (Moeschlin, 1965).

Inorganic arsenite or its anhydride, arsenous oxide, are the most common commercial forms of arsenic. Their acute toxic effects follow a short latent period and include rapid collapse, shock, and death.

Arsenoxides and inorganic pentavalent arsenicals vary considerably in their toxicity. Although they are usually less toxic than the arsenites, their effects on biologic systems appear to be the same.

In general, the phenylarsonic compounds are less hazardous for mammals than other arsenical compounds. The toxic effects of these compounds are manifested by incoordination, inability to control body and limb movements, and ataxia resulting from demyelination of the peripheral nerves (Ledet, 1973).

Arsonium compounds and metallic arsenic are quite stable and have relatively low toxicity (Schroeder and Balassa, 1966; Penrose, 1974).

Toxic Effects in Humans. Human exposure to arsenic sufficient to cause severe toxicosis usually occurs through ingestion of contaminated food or drink. The signs and symptoms are variable in degree and timing and depend on the form and amount of arsenic, the age of the patient, and other factors (Willcox, 1922). The major characteristics of acute arsenic poisoning are profound gastrointestinal damage and cardiac abnormalities.

Symptoms may appear within 8 m if the arsenic is in solution, but may be delayed up to 10 h if it is solid and taken with a meal. The signs include excruciating abdominal pain, forceful vomiting, cramps in the legs, restlessness, and spasms. "A feeble, frequent, and irregular pulse ushers in the other symptoms of collapse, the livid and anxious face, sunken eyes, cold and clammy skin. ...A small proportion of the cases are classed as nervous or cerebral because... the... conspicuous... phenomena are... prostration, stupor, convulsions, paralysis, collapse, and death in coma." (Holland, 1904). Only a small fraction of patients will develop any kind of skin reaction secondary to acute arsenic poisoning. Presumably, the arsenic is absorbed from the damaged gut and finds its way to the skin. The usual reaction in these circumstances is acute exfoliative erythroderma, probably reflecting the fact that arsenic is a capillary poison (Harvey, 1965).

Exposure to amounts of arsenic sufficient to cause symptoms is probably more common than that sufficient to produce systemic collapse. The patient may go for weeks with gradually increasing or variable signs and symptoms related to several organ systems and with the appearance of a progressive chronic disease. If death occurs, it may appear to have been the consequence of an obscure "natural" disease. Skin manifestations of such victims are particularly prominent.

In 1901, over 500 beer-drinkers afflicted with an unusual poisoning attributed to arsenic in one of the ingredients were studied by Reynolds (1901). The symptoms appeared after many months of drinking 2-16 pints per day of beer which contained a fraction of "the quantity of arsenic which [would be prescribed for] an epileptic." Although Frost (1970) has refuted his conclusions and provided evidence that selenium may have been the contaminant, the clinical manifestations described were compatible with those produced by arsenic. The first symptoms to appear were digestive, especially vomiting and diarrhea. Within a few weeks, catarrhal symptoms appeared - such as conjunctivitis, rhinitis, laryngitis, bronchitis - as well as various skin eruptions. hoarseness due to thickening of the vocal chords and hemoptysis were also mentioned. Insidious development of neurologic signs and symptoms began before the appearance of the classical skin lesions, but sometimes were so vague as to go undiagnosed for many weeks. Involvement of the nervous system began with sensory changes, including paresthesias, hyperesthesias, and neuralgias. There was marked muscle tenderness; motor weakness of all degrees (including paralysis with muscle atrophy, progressing from distal to proximal groups was a frequent observation. Left-side heart failure with severe peripheral edema was observed in one-fourth of the patients, and the 13 deaths in this series were all due to congestive heart failure. Reynolds also described the nail changes of subacute arsenic poisoning, observable some weeks after the intake of the poison was stopped. When normal nail grew out, it revealed "transverse white ridge across the nail; proximal to this the nail is normal, but distal to it the nail is whiter, cracked, thin, and towards the tip also papery and much flattened. In some cases there have been a series of parallel transverse ridges on the nail almost suggesting a series of weekend drinking bouts." This feature of arsenic exposure, commonly called "Mees lines" on the basis of a later description, has also been described by Aldrich (1904).

Mitsuta *et al.* (1956) reported on 220 patients of all ages who had been poisoned by contaminated soy sauce, with an average estimated ingestion of roughly 3 mg of arsenic (probably as calcium arsenate) daily for 2-3 weeks. In this group, 85% had facial edema and anorexia; fewer than 10% had exanthemata, desquamation, and hyperpigmentation; and about 20% had peripheral neuropathy. Except for headaches and fever, the findings in these patients appeared to be very similar to those reported by Reynolds (1901). The Japanese report offered additional information based upon modern diagnostic techniques. Although the majority of patients' livers were enlarged, relatively few abnormalities were found in liver-function tests; and the histopathologic description of five liver biopsies did not reveal severe degenerative changes. There were no findings suggestive of congestive failure, but electrocardiograms were abnormal in 16 of 20 patients, and this confirmed the reports of Josephson et al. (1951) and Nagai et al. (1956). The Japanese patients' symptoms tended to diminish after 5 or 6 days, despite continued intake of arsenic, and neurologic symptoms became prominent as much as 2 weeks after arsenic ingestion was discontinued, at which time urinary arsenic content remained high. Hair was found to contain arsenic at 2.8-13.0 ppm near the root, compared with 0-1.5 ppm near the end and 0.4-2.8 ppm in hair from control patients.

In the early 1960's , physicians in Antofagasta, Chile, noted dermatologic manifestations and some deaths, particularly among children, that were traced to a water supply containing arsenic at 800 ppb. This water supply had been in operation only since 1958. In 1971, Borgono and Greiber (1972) reported on a series of studies of the inhabitants of this city. They compared 180 inhabitants of Antofagasta with 98 people who lived in a city (Iquique) with a normal water supply. Most of the people studied were less than 10 years old. Among the residents of Antofagasta the primary symptoms reported were abnormal skin pigmentation (80%); chronic coryza (60%); hyperkeratosis (36%); various cardiovascular manifestations, i.e., Raynaud's syndrome (30%) acrocyanosis (27%); abdominal pain (39%) chronic diarrhea (7%); and lip herpes (13%). The incidence of these symptoms in the control population was substantially lower or nonexistent.

Two additional reports on the Antofagasta studies are worthy of note. Zaldivar (1974) further described a study on a total of 457 patients (208 males, 249 females) bearing cutaneous lesions (leukoderma, melanoderma, hyperkeratosis, squamous-cell carcinoma). Children (0-15 years of age) accounted for 69.2% of male cases, and for 77.5% of female cases. These patients exhibited high arsenic content in the hair. The mean concentration of arsenic in drinking water in the period 1958-1969 was 580 ppb versus 80 ppb in 1971, differing by a factor of 7.2. Such difference was attributed to a new filter plant which started operation in May 1970. The average incidence rates per

100,000 population for cases with cutaneous lesions in 1968-1969 were 145.5 for males and 168.0 for females. The incidence rates decreased in 1971 to 9.1 for males and 10.0 for females.

Among the 337 registered children, 5 died showing thrombosis of brain arteries, thrombosis of mesenteric artery, restriction of lumen of coronary arteries, and/or myocardial infarction. Of the 64 registered adult males, 2 developed multiple skin carcinomata with lymph node metastases.

A number of questions are raised regarding this report. For example, the decrease in cutaneous lesions seemed to be too rapid, following installation of the water treatment plant, suggesting other factors were involved. Protection of the 8-10 year old age group showed up in three years and adults exposed for more than 15 years also had a decrease in incidence rate of cutaneous lesions.

In a follow up study, Borgono et al., (1976) investigated clinical and epidemiologic aspects of the cases first reported in 1971. The study was carried out through the examination of arsenic content in hair and nail clipping samples of the inhabitants of Antofagasta and the determination of this element in cultivated vegetables and carbonated beverages. Also a clinical study was made in school children, looking for cutaneous lesions attributed to arsenicism. Six years after the water treatment plant started to operate the problem had diminished considerably. Arsenic determination of hair and nails of children six years of age or less, born since the water treatment plant went into operation indicated no cutaneous lesions in this age group. However, those over six years of age still had significant arsenic residues in hair and nails. Although the clinical manifestations have improved, arsenic content of water, soft drinks and in some foods are still considerably above safe levels and require additional sanitary engineering improvements.

The Raynaud's phenomenon and acrocyanosis in this population are reminiscent of the report from Taiwan by Tseng et al. (1968) suggesting that chronic arsenism affects the vasculature in a way similar to the more acute phenomena described by Reynolds and others as erythromelalgia and acrocyanosis. Tseng et al. surveyed a group of 40,421 (from a population "at risk" of 103,154) and found hyperpigmentation in 18.4%, keratotic lesions in 7.1%, and blackfoot disease (apparently secondary to arterial spasm in the legs) in 0.9%. All these phenomena were shown to increase with increasing arsenic concentration in the well water of the 37 villages studied. They also increased with age, but the earliest ages noted for specific findings were 3 years for the characteristic hyperpigmentation and 4 years for keratoses. The concentration of arsenic in the wells ranged from 17-1097 ppb. No cases of melanosis or keratosis were found in a group of 2,552 people living in an area where the wells contained almost no arsenic.

Feinglass (1973) reported on 13 persons exposed for 2.5 months to well water contaminated with buried arsenical insecticide. Most patients were seen only once, and the most prominent features were intermittent gastrointestinal symptoms related to water ingestion. Two of the 13 had nail changes, and six to eight had increased arsenic content of the scalp hair. The author did not mention edema, exanthema, hyperpigmentation, or hyperkeratosis.

There are many scattered case reports of subacute to chronic arsenic poisoning in the literature. Silver and Wainman (1952) described a patient who ingested approximately 8.8 mg of arsenic trioxide as Fowler's solution daily for a total period of 28 months, as a remedy for asthma. Signs of arsenic poisoning, manifested as increased freckling and as darkening of the nipples, first appeared in association with gastrointestinal symptoms after 13 months; redness and puffiness about the eyes and hyperkeratoses developed at approximately 1.5 years. Neurologic symptoms in the form of paresthesias and weakness were the last to be noted, occurring after 2 years. When the arsenic intake was stopped, the pigmentation lightened, but the hyperkeratoses remained, and the asthma became more difficult to control.

Perry et al. (1948) noted that all of a group of chemical workers handling inorganic arsenic compounds had pigmentary changes and that one-third of them had "warts," although these were not well described. They reported that the cutaneous "changes were so evident that (the examiner) could readily tell whether the man...was a chemical worker." All these workers had increased urinary arsenic compatible in degree with the extent of exposure; this indicates systemic absorption of the arsenic from dust, probably through the lungs and skin.

Some clinical and experimental evidence suggests that arsenic has the capacity to suppress the immune response selectively. For many medical conditions for which arsenic was most popular, steroid drugs are now the treatment of choice. The high incidence of herpes zoster and herpes simplex in cases of subacute arsenic poisoning is reminiscent of patients who were deliberately immunosuppressed to receive kidney transplants. Recurrent pulmonary infections in children in the Antofagasta episode is reminiscent of children with congenital immunodeficiency syndromes. Arsenic is reputed to reduce the lymphocyte count in leukemia which may reflect a selective sensitivity of this cell type which again is analogous to the effects of steroids (Boryono and Greifer, 1972).

Carcinogenicity. A number of studies in man have linked the appearance of cancer to exposure to inorganic arsenic compounds. Evidence has come from the use of arsenicals as drugs, from geographic areas with high concentrations of arsenic in drinking water, and from arsenic-exposed industrial groups, such as miners and smelters, workers in factories manufacturing arsenic-containing pesticides, and vineyard workers. However, the association of cancer with a history of exposure to arsenic in one form or another must be carefully evaluated before arsenic is incriminated as the causative agent (NAS, 1976).

Evidence of the carcinogenicity of arsenic in man is based almost entirely on descriptive, retrospective, epidemiologic studies. Thus, a change in the rate for cancer in various population groups has been identified, suggesting the influence of carcinogens in the environment of the groups. Although case histories of persons in the afflicted groups have shown exposure to an arsenical, the many variables to which man is subject cannot be controlled in retrospective studies. In none of the human studies was there satisfactory control of exposure to known carcinogens (including cigarette smoke, asbestos, ionizing radiation, polycyclic hydrocarbons, pesticides, and ultraviolet light) or other unknown carcinogens in the environment.

The clinical association of skin cancer with the therapeutic administration of arsenic compounds began with a report by Hutchinson (1888). Six patients in whom skin cancer occurred had suffered for very long periods from diseases of the skin (five with psoriasis, one with pemphigus). In five of the cases, arsenic was known to have been used for a long time. Neubauer (1947) summarized 143 published cases of therapeutic arsenical epitheliomas. Only a small, but undetermined, proportion of people treated with arsenicals developed cancers. Of the 143 patients, about 70% received arsenicals for skin disease; of these, half had psoriasis. Nearly all the 143 patients received arsenic in the inorganic trivalent form, the most common drug being potassium arsenite as Fowler's solution. Multiple horn keratoses, especially of the punctate or warty form on the palms and soles, were commonly reported in patients who had received Fowler's solution. Keratoses occurred in about 90% of the cases of cancer ascribed to treatment with Fowler's solution; about half the skin cancers were squamous carcinomas arising in keratotic areas of the hands, heels, and toes. The rest were multiple superficial epitheliomas of the basal-cell variety localized on the trunk and proximal parts of the extremities. Only a few of the 143 cases arose in psoriatic patches. There was a substantial frequency of mixed types of epitheliomas. Of the 141 patients, 70% had multiple lesions, with an average of two per case. The elapsed time from the beginning of administration of the arsenical drug to the beginning of the epitheliomatous growth was variable, but averaged 18 years, regardless of the type of lesion. The latent period for the onset of keratosis was about 9 years. In spite of the long latent period, skin cancers started when the patients were relatively young, 30% when they were 40 or younger and 70% when they were 50 or younger.

There have been numerous reports of arsenic-related occupational cancer, such as those of lung-cancer mortality among Southern Rhodesian gold miners (Osburn, 1957) and of concurrent lung and liver cancer and clinical arsenism among German vineyard workers exposed to lead arsenate dust (Braun, 1958; Roth, 1957). The association of cancer with arsenic has sometimes been based on the existence of palmar or plantar keratoses (Sommers and McManus, 1953). However, because of the increased concentration of arsenic in the lesions of Bowen's disease, arsenic has been considered as a possible cause of the disease and accompanying visceral tumors, without prior exposure to arsenicals (Graham *et al.*, 1961).

Hill and Faning (1948) compared the death records of workers in a British sheep-dip factory with those of other workers in the same district. Of the 75 factory-workers deaths, 22 (29%) were due to cancer; of the 1,216 other deaths, 157 (13%) were due to cancer. The sites of cancer were primarily the respiratory system and the skin in the factory workers, and there was a considerable incidence of cancer of digestive and abdominal organs in both factory workers and other occupational groups. The median air arsenic content for the chemical workers at the various operations ranged from 254-696 μg/m³. As an upper limit, this represented inhalation of about 1 g of arsenic per year. The excretion of arsenic in the urine of 127 current employees was determined and varied widely. Some exposed workers excreted from 1 to nearly 2 mg/day, whereas many excreted less than 0.1 mg/day. A few of the persons in the control group had very high excretion rates, for no explained reason. It is important to note that 20 of 31 current factory workers had been exposed to airborne sodium arsenite for more than 10 years, and five of them for 40-50 years. Furthermore, the median age of the 31 exposed workers was 52 years. None of these men's lungs had pathologic signs attributable to their exposure to sodium arsenite (radiographs, vital capacity, and exercise capacity were studied). The authors concluded that the study of factory workers had produced no concrete evidence to confirm the association of arsenic exposure to death from cancer.

Lee and Fraumeni (1969) compared the mortality experience of 8,047 white male smelter workers exposed to arsenic trioxide and sulfur dioxide during 1938-1963 with that of the white population in the same state. There was a threefold greater total mortality from respiratory cancer in smelter workers, many of whom were also exposed to silica, ferromanganese dust, and and other fumes.

Snegireff and Lombard (1951) made a substantial study of cancer mortality in a metallurgic plant in which arsenic was handled and in a control plant in which "working conditions approximate those of Plant A except that no arsenic is handled." The authors stated that total cancer mortality in the two plants was not significantly different from the figures for the state as a whole, and they conluded that handling of arsenic trioxide did not cause a significant change in cancer mortality. Their data demonstrated, however, evidence of a respiratory system carcinogen among workers of both plants, but arsenic may not have been involved.

A study at the Dow Chemical Company was carried out to examine the incidence of respiratory cancer among 173 workers who were exposed primarily to lead arsenate and calcium arsenate and 1,890 workers who worked in the same plant but were not exposed to arsenic (Ott et al., 1974). Data were presented on the relationship between cumulative arsenic exposure and the ratio of observed to expected deaths from lung cancer. The average exposure of each worker was calculated on the basis of records of job assignments and data on the arsenic content of the air in various parts of the plant. Deaths from respiratory malignancy were 6-7 times greater than expected for total inhaled quantities of 10.1 mg and 2-4 times greater for 4.84-8.17 mg. There was no association between the extent of exposure and the time from beginning of exposure to death; most of the respiratory cancers occurred 20-40 years after initial exposure, regardless of total exposure.

In contrast with the Dow Chemical Company workers, orchard workers who used lead arsenate had no evidence of increased cancer (Nelson et al., 1973). A mortality study involving a cohort of 1,231 workers in Wenatchee, Washington, who had participated in a 1938 morbidity survey of the effects of exposure to lead arsenate insecticide spray was conducted in 1968-1969. Air concentrations of arsenic during spraying averaged 0.14 mg/m^3. The workers were grouped in three categories according to exposure and compared in terms of standardized mortality ratios with the mortality experience of the State of Washington. There was no evidence of increased mortality from cancer, heart disease, or vascular lesions.

High incidences of skin cancer have been reported in several groups exposed to high concentrations of arsenic in drinking water, including people in the district of Reichenstein in Silesia (Geyer, 1898), Cordoba Province in Argentina (Bergoglio, 1964), and Taiwan (Tseng et al., 1968).

The existence of arsenic in waters in an eastern area of the province of Cordoba, Argentina, has been known for many decades; it has been associated with the occurrence of hyperpigmentation, keratosis, and skin cancer. A study made in 1949-1959 indicated a higher proportion of deaths from cancer in the arsenical region than in the rest of the province--23.8% vs. 15.3% (Bergoglio, 1964). The excess was due mainly to cancer of the respiratory and digestive tracts in both men and women and was unrelated to socioeconomic differences.

A study by Tseng et al. (1968) was done on the southwest coast of Taiwan, where there were artesian wells that had been used for more than 45 years with high concentrations of arsenic. Most of the well water in the endemic area had an arsenic content of around 0.5 ppm. The total population of the area was approximately 100,000, and the survey encompassed the 40,421 inhabitants of 37 villages. The overall prevalence rates for skin cancer, hyperpigmentation, and keratosis were 10.6/1,000, 183.5/1,000, and 71.0/1,000, respectively. The male:female ratios were 2.9:1 for skin cancer and 1.1:1 for hyperpigmentation and keratosis. The prevalence of each of the three conditions increased steadily with age, although there was a decline for cancer and hyperpigmentation in women above 69. The prevalence rate for each condition varied directly with the arsenic content of the well water.

In a continuing survey and follow-up in some villages of Taiwan, Tseng (1976) confirmed that the prevalence rates for skin cancer and Blackfoot disease showed an ascending gradient according to the arsenic content of well water, i.e. the higher the arsenic content, the more patients with skin cancer and Blackfoot disease. A dose-response relationship between Blackfoot disease and the duration of water intake was also noted. Furthermore, the degree of permanent impairment of a patient was noted to be directly related to duration of intake of arsenical water and alternatively to duration of such intake at the time of onset. The most common cause of death in the patients with skin cancer and Blackfoot disease was carcinoma of various sites. 5-y survival rate after Blackfoot onset was 76.3%, 10-y survival rate, 63.3%, and 15-y survival rate, 52.2%. The 50% survival point was 16 years after onset of the disease.

The more recent observations from Chile and from Taiwan emphasize the public health problems associated with arsenic in specific geographic areas.

Recent reports from Sweden (Pershagen et al., 1976) are of interest. The study concerns mortality in an area surrounding an arsenic emitting plant. A metallurgic plant, founded in 1928, processed mainly nonferrous metals. Since the starting of operations it has been using ore with a high arsenic content which has resulted in environmental pollution of air and water with arsenic, as well as other metals and sulphur dioxide.

The causes of death for the population of two parishes in the vicinity of the plant were listed from the National Swedish Register on Death Causes. A reference area in the same part of Sweden with similar degree of urbanization, occupational profile, and age distribution was chosen. The causes of death for the two populations were followed during a period of fourteen years. A markedly higher mortality rate for lung cancer was noted in men in the exposed area. Also when the occupationally exposed were excluded there remained indications of an increased mortality in men due to primary respiratory cancer.

A continuation of this investigation in the form of a cohort study will consider both the mortality and cancer incidence.

Kenzaburo (1976) has recently reported from Japan a number of incidents of arsenic poisoning associated with a variety of vehicles including powdered milk, soy sauce, well water, mines and smelters. Effects varied according to the dose, duration, and route of exposure. In the milk incident, infants were exposed to relatively high doses of arsenic in powdered milk and the victims developed acute symptoms of the gastrointestinal tract; in some cases, symptoms of the central nervous system, anemia, neuropathy, cardiovascular and skin changes, but no neoplasms. It is not clear whether the symptoms of the central nervous system were due to the stimulation of the cerebral membrane or to organic changes of the cerebral parenchyma. It is important to note that the development of some possible changes of the brain as indicated by EEG and possibly by the higher incidence of epilepsy occurred at a later stage--as late as 15 years after clinical changes had disappeared. No other study has reported the development of chronic encephalopathy among heavily exposed children or adults.

Another important question after having reviewed these episodes is whether arsenic is related to the causation of liver cirrhosis. In incidents reported in Japan, there is no increased prevalence of liver cirrhosis among those exposed to arsenic. In the well water incident, the married couple who had been drinking water containing 0.125 ppm arsenic showed liver cirrhosis (the husband) and Banti syndrome (the wife). However, since no cases of either disease have been observed among those who suffered heavier exposure, the relationship between these diseases and arsenic is still open to question.

In the soy sauce incident, it was noted that the symptoms improved even while the ingestion of the contaminated soy sauce was still in progress. The mechanism of this phenomenon should be further investigated.

The report on the increased prevalence of abnormal emg findings is also of interest since prolongation of electric conduction velocity has been reported in persons whose blood lead level was lower than 70 mg/100 ml.

Since there have been no other reports on the increased risk of lung cancer due to arsenic among occupationally exposed workers in Japan, and also since the induction of lung cancer by arsenic in animal experiments has not been successful, the direct relationship between arsenic and lung cancer is still open to question. In the Saganoseki copper smelter incident, attention was drawn to the fact that smelter workers had also been exposed rather heavily to substances other than arsenic, including polynuclear organic substances, sulfur dioxide and possibly to other chemical substances.

Mutagenicity. Petres and Berger (1972) and Petres and Hundeiker (1968) have reported chromosomal breakage in human leukocyte cultures after short-term in vitro exposure to sodium arsenate and in cultures obtained after long-term exposure to arsenical compounds in vivo. The cytotoxic and mutagenic effects of sodium arsenate were tested in vitro on phytohemagglutinin-stimulated lymphocyte cultures in concentrations of 0.05-30 μg/ml of culture medium. It was reported that 13% of metaphase plates were disrupted at 0.1 μg/ml and 80-100% at 2 μg/ml or greater. The "mitosis index" and the "(^{3}H) thymidine labelling index" were decreased.

Petres et al. (1976) did chromosome analyses of lymphocytes from patients who had been exposed to arsenic. They showed frequent structural and numerical aberrations even following an interval of decades since the last exposure.

The in vitro addition of sodium arsenate induced the same chromosome changes in lymphocyte cultures from healthy subjects. Radioactive incorporation studies showed that arsenate was able to inhibit dose-dependently the incorporation of radioactively-labeled nucleotide in RNA and DNA. Beyond that, arsenic blocked the cells in the S- and G-phase.

A general explanation for the inhibitory effect of inorganic arsenic on cell metabolism is the known strong affinity of arsenic to enzymes, especially to those containing sulfhydryl-groups. These studies require attention and further investigation.

The overall significance of these chromosomal studies is difficult to assess, because many unrelated compounds may cause similar effects. The fact that arsenic compounds have caused chromosomal damage in a number of biologic systems, however, should alert toxicologists to a possible role of arsenic in chemically induced mutagenesis.

In vivo studies were made on 34 patients at the University of Freiburg skin clinic (Petres _et al_., 1970). Thirteen of these patients had had intensive arsenic therapy for psoriasis, some more than 20 years before the experiment. The control group (21 patients) consisted of 14 psoriasis patients and seven with eczema, none of whom had had arsenic treatment. Phytohemagglutinin-stimulated lymphocyte cultures were prepared from each patient for evaluation of chromosomal aberrations. The incidence of aberrations was remarkably greater in the cultures of patients who had been treated with arsenic.

Paton and Allison (1972) investigated the effect of sodium arsenate, sodium arsenite, and acetylarsan on chromosomes in cultures of human leukocytes and diploid fibroblasts. Sublethal doses of the arsenicals were added to leukocyte and fibroblast cultures at varous times between 2 and 48 h before fixation. In leukocyte cultures treated with sodium arsenite at 0.29-1.8 X 10^{-8} M for the last 48 h of the culture period, 60% of 140 metaphases examined were found to have chromatid breaks. No significant number of breaks were found in cultures treated with sodium arsenate at 0.58 X 10^{-8} M, the highest nontoxic concentration. Chromosomal damage was observed in diploid fibroblasts to which sodium arsenite was added to the medium for the last 24 h of culture; chromatid breaks were found in 20% of 459 metaphases examined. However, treatment with acetylarsan at 6.0 X 10^{-8} M resulted in 20% chromatid breaks in 50 metaphases examined.

Environmental exposure to arsenicals has been correlated with a high skin cancer risk among populations exposed to sunlight. These observations suggest that arsenic might interfere with the repair of damage to DNA (mostly thymine dimers) resulting from the ultraviolet rays in sunlight. To test this hypothesis Rossman _et al_. (1976) have used strains of _E. coli_, differing from each other only in one or more repair fuctions, to study the interactions. Cultures of _E. coli_ were exposed to UV light and then plated in the presence or absence of sodium arsenite.

survival after irradiation of wild type *E. coli* (WP2) was significantly decreased by 0.5mM arsenite. This effect was also seen in strains unable to carry out excision repair, suggesting that arsenite inhibits one or more steps in the post-replication repair pathways. This was confirmed by the finding that arsenite has no effect on the post-irradiation survival of a *recA* mutant, which does not carry out postreplication repair.

Mutagenesis after UV-irradiation depends on the recA+ and lex+ genes. Arsenite decreases mutagenesis in strains containing these genes. In order to determine its mechanism of action, Rossman *et al.* studied dose-response relationships of arsenite on a number of cellular functions. The most sensitive cellular functions found were the induction of β-galactosidase and the synthesis of RNA. Since error-prone repair in *E. coli* is an inducible process, the inhibition of mutagenesis after UV irradiation may be the result of inhibition of messenger RNA.

Since arsenite inhibits DNA repair in *E. coli*, specifically post-replication repair, this may be a possible mechanism through which it influences the induction of cancer.

Toxic Effects in Animals. Arsenic appears to be second only to lead in importance as a toxicant in farm and household animals (Buck *et al.*, 1973; Hatch and Funnell, 1969). Some of the more common sources of arsenic poisoning include grass clippings from lawns that have been treated with arsenical crabgrass control preparations; grass, weeds, shrubbery, and other foliage that have been sprayed with arsenical herbicides (Buck, 1969); dipping of animals in vats that even years before had been charged with arsenic trioxide; and soils heavily contaminated with arsenic, either from the burning of arsenic formulations in rubbage piles or through the application of arsenical pesticides to orchards and truck gardens (Clarke and Clarke, 1967; Radeleff, 1970). Arsenical compounds dissolved in water are much more readily absorbed, and thus more toxic than when incorporated into feed (Buck *et al.*, 1973).

In practice, the most dangerous arsenical preparations are dips, herbicides, and defoliants, in which the arsenic is in a highly soluble trivalent form, usually arsenite. Animals often seek out and eat such materials as insulation, rodent bait, and dirt and foliage that have been contaminated with arsenic (Buck, *et al.*, 1973).

Animals that are weak, debilitated, and dehydrated are much more susceptible to arsenic poisoning than normal animals, probably because renal excretion is reduced.

Arsenic poisoning in most animals is usually manifested by an acute or subacute syndrome. Arsenic affects tissues that are rich in oxidative systems, primarily the alimentary tract, kidneys, liver, lungs, and skin. It is a potent capillary poison; although all capillary beds may be involved, the splanchnic area is most commonly affected. Capillary damage and dilatation result from transudation of plasma into the intestinal tract and from sharply reduced blood volume. The capillary transudation of plasma results in the formation of vesicles and edema of the gastrointestinal mucosa, which eventually lead to epithelial sloughing and discharge of the plasma into the gastrointestinal tract. (Radeleff, 1970). Blood pressure usually falls to the point of shock, and cardiac muscle becomes weakened; these effects contribute to circulatory failure.

Toxic arsenic nephrosis is more commonly seen in small animals and man than in farm animals. Glomerular capillaries dilate, allowing the escape of plasma; this results from the loss of fluid through other capillary beds, and the low blood pressure contributes to the oliguria that is characteristic of arsenic poisoning. The urine usually contains protein, red blood cells, and casts (Buck *et al.*, 1973).

After percutaneous exposure, capillary dilatation and degeneration may result in blistering and edema, after which the skin may become dry and papery. The skin may then crack and bleed, providing a choice site for secondary bacterial invaders (Radeleff, 1970).

In subacute arsenic poisoning, animals may live for several days and show depression, anorexia, watery diarrhea, and increased urination followed by anuria, dehydration, thirst, partial paralysis of the rear limbs, trembling, stupor, coldness of extremities, and subnormal temperature. The stools may contain shreds of intestinal mucosa and blood.

Characteristic gross effects associated with inorganic, aliphatic, and aromatic trivalent arsenic poisoning include localized or general reddening of the gastric mucosa, reddening of the small intestinal mucosa (especially the first few feet of the duodenum), fluid and often foul-smelling gastrointestinal contents, a soft yellow liver, and red edematous lungs. Occasionally, in acute poisoning, no gross changes are noted post mortem. The inflammation is usually followed by edema, rupture of the blood vessels, and necrosis of the mucosa and submucosa. This necrosis sometimes progresses to perforation of the stomach or intestine. The gastrointestinal contents may include blood and shreds of mucosa. There may occasionally be hemorrhages on all surfaces of the heart and on the peritoneum (Clarke and Clarke, 1967).

Histopathologic changes include edema of the gastrointestinal mucosa and submucosa, necrosis and sloughing of mucosal epithelium, renal tubular degeneration, hepatic fatty changes and necrosis, and capillary degeneration in vascular beds of the gastrointestinal tract, skin, and other organs (Buck *et al.*, 1973; Radeleff, 1970).

The work of Schroeder *et al.* (1968) and Peoples (1964) indicates that the rat may be unique in its susceptibility to and metabolism of arsenic compounds. Schroeder *et al.* noted that, although rats consuming water containing arsenite at 5 ppm accumulated arsenic at 27-47 ppm in their body tissues, they developed no signs of toxicosis and survived a normal 3.5 year life span. Peoples showed that the rat is unique in its low rate of excretion of arsenic. Both reported that the blood of rats tended to accumulate high concentrations (100-300 ppm) of arsenic. This is not observed in humans. The low rate of arsenic excretion by the rat is probably due to fixation of 80-90% in the hemoglobin, which must break down before arsenic is released. These experiments are sufficient to cast doubt on the extrapolation of data from arsenic experiments involving rats to man.

Several phenylarsonic formulations have been used as feed additives for disease control and improvement of weight gain in swine and poultry since the mid-1940's. These phenylarsonic acids and their salts include arsanilic acid, 3-nitro-4-hydroxyphenylarsonic acid, 4-nitro-phenylarsonic acid, and 4-ureido-1-phenylarsonic acid.

There remains considerable controversy regarding the mode of action of the phenylarsonic compounds. However, they seem to have an action different from that of inorganic, aliphatic, and aromatic trivalent arsenic compounds. The arsenic incorporated in the feed additives is in the pentavalent form, and it is likely that the phenylarsonic compounds have their primary action as pentavalent arsenicals; this may account for their distinctive effects in birds and animals. Some workers have suggested that both the toxicity and the efficiency of these compounds are due to their degradation and reduction to inorganic trivalent forms (Eagle and Doak, 1951; Harvey, 1965; and Voegtlin and Thompson, 1923). Others have clearly established that these compounds are excreted unchanged by chickens and that there is no evidence that they are converted to inorganic arsenic (Moody and Williams, 1964a; Moody and Williams, 1964b; Overby and Fredrickson, 1963; 1965, and Overby and Straube, 1965). Similar experiments by other workers with rats, rabbits, and swine indicate that the phenylarsonic compounds are for the most part excreted unchanged by the kidneys, although some apparently undergo a limited biotransformation (Moody and Williams, 1964b).

Because pentavalent arsenic compounds do not readily react with sulfhydryl groups and because the phenylarsonic acids are apparently excreted unchanged, one must conclude that the mechanism of their action is something other than interaction with sulfhydryl containing enzymes and proteins.

Clinical signs of phenylarsonic toxicosis in swine and poultry include incoordination, inability to control body and limb movements, and ataxia. After a few days, swine and poultry may become paralyzed, but will continue to eat and drink. Arsanilic acid and its sodium salt may produce blindness but this is rarely seen when it is used as one of the most common feed additives. Erythema of the skin, especially in white animals, and sensitivity to sunlight may be present. The clinical signs are reversible up to a point. Removing the excess arsenical will result in recovery within a few days, unless the clinical signs have progressed to partial or complete paralysis due to irreversible peripheral nerve degeneration (Buck, 1969b; Oliver and Roe, 1957).

Chronic arsenic toxicosis has not been encountered significantly in animals.

Gainer and Pry (1972) reported that virus-infected mice treated subcutaneously with large doses of arsenicals had higher mortality rates than unexposed controls. Viral diseases so affected by arsenic were pseudorabies, encephalomyocarditis, and St. Louis encephalitis. The effects were similar when 3-nitro-4-hydroxyphenylarsonic salt (75-100 ppm) was added to drinking water. Gainer (1972) reported that sodium arsenite inhibited the induction of interferon in rabbit kidney cell cultures. It was found, however, that, although high concentrations of arsenite inhibited the action of exogenous mouse interferon added to cultures of mouse embryo cells, low concentrations of arsenite increased the antiviral activity of low concentrations of interferon.

Carcinogenicity. Animal studies have not demonstrated carcinogenicity of arsenic compounds even when administered at near the maximal tolerated dosage for long periods. There are two exceptions, however. Halver (1962) reported the occurrence of hepatomas in trout fed a synthetic diet containing carbarsone at 480 mg/100 g of diet. The data were reviewed by Kraybill and Shimkin (1964). Of 50 trout exposed to carbarsone, five developed hepatomas. There were no hepatomas in a large control group fed the synthetic diet without carbarsone. Osswald and Goerttler (1971) reported that subcutaneous injections of sodium arsenate in pregnant Swiss mice caused a considerable increase in the incidence of leukemia in both the mothers and their offspring. A 0.005% aqueous sodium arsenate solution was injected daily during pregnancy for a total of 20 injections, each containing arsenic at 0.5 mg/kg of body weight. Some groups of offspring from the arsenic-treated females were given an additional 20 subcutaneous injections of sodium arsenate (arsenic equivalent 0.5 mg/kg) at weekly intervals. Leukemia occurred in 11 of 24 mothers (46%), seven of 34 male offspring (21%), and six of 37 female offspring (16%). In the offspring given the additional 20 injections, 17 of 41 males (41%) and 24 of 50 females (48%) developed leukemia. Leukemia developed in only three of 35 male (9%) and in none of 20 female offspring of untreated control mice. Furthermore, 11 of 19 mice (58%) developed lymphoma after 20 weekly intravenous injections, each containing 0.5 mg/kg of arsenic as sodium arsenate.

Long-term studies of effects of arsanilic acid on chickens, pigs, and rats were reported by Frost et al. (1967). No adverse effects were seen in the chickens and pigs after 4 years of feeding, nor in pigs fed 0.01% arsanilic acid in their diets for three generations. Male and female weanling rats from the F2 generation of a six-generation breeding study in which 0.01% and 0.05% arsanilic acid was fed were held on the 0.01% arsanilic acid diet or on the control diet for 116 weeks. The overall tumor incidence was the same in all groups and resembled the historical incidence of tumors in the colony, 35-45%.

Boutwell (1963) used female mice known to be highly susceptible to skin tumors in a test for cocarcinogenicity of potassium arsenite. It was tested as an initiator, both orally by stomach tube (a total of 2.4 mg in 5 days) and dermally (a total of 1.2 mg in eight applications during 5 days). The initiating exposure was followed by topical application of croton oil twice a week for 18 weeks. He also tested potassium arsenite as a promoter by daily applications (a total of 2.3 mg/w) after a single 75 μg dose of dimethylbenzanthracene (DMBA). The prolonged skin applications of potassium arsenite were hyperkeratotic and ulcerogenic. Other experiments were done to determine whether arsenic would increase the yield of skin cancers caused by a suboptimal regimen of DMBA plus croton oil given either at the time of DMBA initiation or during the 24-w period of croton oil promotion. Under the latter condition, the mice were fed potassium arsenite at 169 mg/kg of food. In no case was there an effect of arsenite on skin carcinogenesis in these experiments. Many tumors developed in the positive control mice, beginning as early as 6 weeks after treatment began.

Baroni et al. (1963) conducted similar studies with male and female Swiss mice, testing the oral effects of potassium arsenite (100 ppm in drinking water) as an initiator with croton oil promotion and as a promoter for DMBA and urethane initiation.

Local skin applications of sodium arsenate were tested as a promoter after initiation with DMBA or urethane. The arsenicals had no effect on carcinogenesis, and only a very slight degree of keratosis was observed.

Milner (1969) used three strains of mice that differed in susceptibility to the induction of skin tumors with the application of methylcholanthrene-impregnated paraffin disks to the skin for 2-3 weeks. The treated site was transplanted syngeneically and observed for 8 w for tumor formation. Arsenic trioxide (100 ppm in drinking water) was administered either during methylcholanthrene exposure, after transplantation, or both. Arsenic exposure was associated with a small increase in papillomas in the low-susceptibility strain, a small decrease in the high-susceptibility strain, and no effect in the intermediate-susceptibility strain.

Byron *et al.* (1967) fed sodium arsenite at 15-250 ppm and sodium arsenate at 30-400 ppm to Osborne-Mendel rats in a 2-y study. No carcinogenic activity of either material was found. These investigators also did a 2-y arsenic feeding experiment on dogs, with negative results. This length of time, however, is not adequate for studying carcinogenesis in dogs.

Hueper and Payne (1962) incorporated arsenic trioxide in the drinking water (either plain or with 12% ethanol) of rats and mice. The initial concentration of 4 mg/liter was increased by 2 mg/liter each month, to a maximum of 34 mg/liter at 15 months. Thus, the daily intake of arsenic trioxide ranged from 0.1-0.8 mg. The administration of arsenic trioxide was continued for 24 months. Neither the rats nor the mice developed any cancers in suspected target organs--skin, lungs, and liver.

Kanisawa and Schroeder (1967) and Schroeder *et al.* (1968) found no carcinogenic effects of potassium arsenite at 5ppm in drinking water in mice or rats exposed from weaning to senescence.

Kroes *et al.* (1974) studied the carcinogenicity of lead arsenate and sodium arsenate with SPF-Wistar-derived male and female rats. In addition, some groups were intubated with a subcarcinogenic dose of diethylnitrosamine to determine synergistic action leading to lung tumors. Food intake and body weights were recorded, and complete gross and microscopic examinations were made on all animals. Lead arsenate that was incorporated in the diet at 1,850 ppm was toxic and caused increased mortality; one adenoma of the renal cortex and one bile duct carcinoma were found in this group. No carcinogenicity was associated with the feeding of lead arsenate at 463 ppm or sodium arsenate at 416 ppm. No synergism with the nitrosamine was observed.

In summary, there is epidemiologic evidence of the carcinogenic action of arsenic on the skin and lungs of humans, on the basis of experience with the medicinal use of inorganic trivalent arsenic, occupational groups exposed to inorganic trivalent or pentavalent arsenic dusts, and populations exposed to high concentrations of arsenic in drinking water. In most instances, however, the exposures to arsenic have been concurrent with exposures to other agents, and the available data do not exclude the possibility that cofactors are important in the carcinogenic response to arsenic. Differences in the type and distribution of tumors, attributed to the ingestion of arsenic, raise serious questions with respect to a simple etiologic relation of arsenic to the various findings. There is no established procedure to demonstrate carcinogenicity of arsenic in experimental animals. This phenomenon remains an enigma. One must conclude either that arsenic is not a carcinogen for animals or that circumstances not yet understood are essential to demonstrate a role for arsenic in experimental carcinogenesis.

Mutagenicity. Most of the research on mutagenesis of arsenic has centered on chromosomal reactions to sodium arsenate. There are no data based on the host-mediated assay or the dominant-lethal technique.

Levan (1945) treated root meristem cultures of *Allium cepa* for 4 h with an unspecified arsenic salt at 10 concentrations, from lethal to no-effect. Chromosomal changes were observed, including spindle disturbances and metaphase arrests. Similar effects were observed after treatment with salts of 24 other metals.

Arsenate has also been found to increase the total frequency of exchange chromosome in *Drosophila melanogaster* treated with selenocystine (Walker and Bradly, 1969), and several organic arsenicals have a synergistic effect on the number of abnormalities in barley chromosomes caused by ethylmethane sulfonate (Moutschen and Degraeve, 1965).

Teratogenicity. Franke *et al.* (1936) performed what might be called the first teratogenic study of an arsenic compound, when they tested the effect of sodium arsenite on the development of chick embryos. Injection of sublethal concentrations of arsenic into the eggs produced ectopic conditions, but no monstrosities, as are produced by selenium.

Ridgway and Karnofsky (1952) found that injection of sodium arsenate into embryonate chicken eggs at 4 days in doses of 0.20

mg/egg caused no specific gross abnormalities in the resulting embryos 14 days later. Growth retardation, impaired feather growth, and abdominal swelling were noted.

Recent studies have demonstrated teratogenic effects of intravenous administration of sodium arsenate in mice and hamsters (Ferm and Carpenter, 1968; Ferm *et al.*, 1971). Single doses (15-20 mg/kg) were administered on the eighth day of gestation, and the results were observed on the fifteenth day; there was a high incidence of anencephaly and other defects. Up to 80% of the embryos had anencephaly; up to 65%, rib malformations; up to 30%, exencephaly; and approximately 20%, genitourinary malformations. Incidences of renal agenesis and cleft lip and palate were lower. Further analysis of the teratogenic consequences of sodium arsenate by Holmberg and Ferm (1969) showed that sodium selenite injected at 2 mg/kg simultaneously with a teratogenic dose of sodium arsenate decreased the number of fetal resorptions and congenital malformations caused by the arsenical.

In mouse studies, Hood and Bishop (1972) administered a single dose of sodium arsenate or arsenite by intraperitoneal injection on a specific day from the sixth to the twelfth day of gestation and observed the results on the eighteenth day. The injections given on the ninth day were most teratogenic; 60% of 96 implantations were resorbed or dead, and 63% were grossly malformed. The defects included exencephaly, micrognathia, protruding tongue, agnathia, open eye, cleft lip, fused vertebrae, and forked ribs. Mice that received injections of distilled water served as controls. Although teratogenic effects were seen at 45 mg/kg, 25 mg/kg was without effect. Sodium arsenite was more effective, which indicated that the extent of fetal anomalies caused by sodium arsenite at 10 mg/kg was comparable with that caused by sodium arsenate at 45 mg/kg. Hood and Pike (1972) reported that BAL, when administered to mice at 50 mg/kg by intraperitoneal injection within 4 h of sodium arsenate at 40 mg/kg, prevented the arsenic-induced teratogenesis.

Potassium arsenate was fed to four pregnant ewes at 0.5 mg/kg during most of pregnancy without effect (James *et al.* 1966).

Interactions. Moxon (1938) first reported the protective effect of arsenic against selenium poisoning when he found that sodium arsenite (5 ppm) in drinking water reduced liver damage in rats on a diet containing selenium at 15 ppm in seleniferous wheat. Moxon and DuBois (1939) demonstrated that arsenic was unique in its ability to prevent selenium toxicity. The protective effect of arsenic against dietary selenium was not seen when the arsenic was added to the diet, instead of the drinking water (Ganther and Baumann, 1962). Frost (1967) reported that selenium and arsenic are additive in toxicity if both are added to the drinking water.

Ganther and Bauman (1962) reported that excretion of selenium into the gastrointestinal tract was markedly stimulated by arsenic when both elements were injected parenterally. Levander and Bauman (1966a,b) showed that this increased selenium excretion occurred through the bile.

Sodium arsenite is the most effective in enhancing biliary excretion of selenium, but arsenate and the phenylarsonates were also somewhat effective. This leads one to question the role of phenylarsonic feed additives in exacerbation of selenium deficiency in animals. Surprisingly, Muth *et al.* (1971) reported that sodium arsenate (1 ppm) added to a selenium-deficient diet significantly reduced the incidence of myopathy in lambs. This work has not been confirmed.

Beneficial Effects. A number of older publications suggested that small amounts of arsenic may have beneficial effects in human and animal health (Underwood, 1971). Until recently, however, there has been no proof of the essentiality of arsenic in mammals. Hove *et al.* (1938) concluded that, if arsenic were essential to the rat, the requirement must be somewhere below 2 µg daily. Schroeder and Balassa (1966) reported that rats and mice grew well when they received only 0.26 µg of arsenic per 100 g of body weight per day in food (0.026 mg/kg). In a recent preliminary report, Nielsen *et al.* (1975) presented evidence that rats require arsenic at about 30 ppb in a synthetic diet. The deficiency signs were rough hair coat, retarded growth, splenomegaly, reduced hematocrit, and increased red-cell fragility.

The phenylarsonic compounds have been used as feed additives for disease control and improvement of weight gain in swine and poultry since the mid-1940's (Bird *et al.*, 1949; Frost, 1967; Morehouse, 1949).

Analysis. Much difficulty has been experienced with chemical analyses for arsenic, especially in biologic samples. Improvements in instrumentation, especially atomic absorption spectrophotometers, have facilitated such analyses somewhat. In most cases, arsenic determinations on drinking water samples are less complicated than those on biologic samples.

Determination of Arsenic in Drinking Water. The current National Interim Primary Drinking Water Regulation for arsenic, 50 µg/liter, is 5 times as great as that for selenium. As was the case for selenium, the *1975 Chemical Analysis of Interstate Carrier Water Supply Systems* (EPA, 1975) indicated that the maximal allowable concentration was seldom exceeded, although in a few instances the method of analysis had a minimal detection limit higher than the standard.

One of the most sensitive methods for arsenic analysis in water is the atomic-absorption procedure of Fernandes (1973). The method has an absolute detection limit of 10 ng, which, for a 20-ml. sample, provides a solution detection limit of 0.5 μg/liter--one twentieth of the acceptable drinking-water standard of 50 mg/liter. The sensitivity can be increased by using an electrodeless discharge lamp, giving an absolute detection limit of 3 ng and a concentration detection limit of 0.15 μg/liter. The method applies to both inorganic and organic arsenic. There are few interference problems with drinking-water samples.

Determination of Arsenic in Biologic Samples. A modification of the Fernandes (1973) atomic-absorption method has been adopted by the National Institute for Occupational Safety and Health (1974) for urine and hair samples. The samples are digested in acid; after acid removal, the arsenic is converted to arsine with metallic zinc or sodium borohydride, $NaBH_4$. The arsine is flushed into the burner of the atomic-absorption spectrophotometer for measurement. As little as 1 μg of arsenic per liter of urine can be detected by this method.

Colorimetric methods can detect as little as 10 μg/liter of urine (National Institute for Occupational Safety and Health, 1974; Horwitz, 1970). The samples are digested as stated above, and the arsine generated reacts with ammonium molybdate, sulfuric acid, and hydrazine sulfate, to develop a chromophore with an absorption peak at 845 nm; or the arsine reacts with silver diethyldithiocarbamate, and the chromophore concentration is determined at 522 nm. (If pyridine is present, an altered chromophore is measured at 560 nm.) At concentrations approaching the arsenic concentration, antimony will interfere with the colorimetric assays.

Conclusions And Recommendations. The evidence for an association between arsenic and disease in some human populations has been further strengthened by recent epidemiological studies such as those conducted in the waters of Puget Sound, in local water supplies such as those in Lassen County, California; Perham, Minnesota; Lane County, Oregon; Antofagasta, Chile and on the southwest coast of Taiwan. Skin lesions, including cancer, and a circulatory disorder referred to as "Blackfoot" are major clinical problems where chronic exposure to arsenic exists. Human disease associated with arsenic is not exactly duplicated in animals although misuse of arsenicals results in disease in dogs and in cattle. There is no animal model for study of arsenic-induced cancer. Arsenic causes fetal death at high doses and malformations at lower exposure in hamsters, mice and rats. Bacterial systems have revealed that arsenic interferes with DNA repair. The different forms of arsenic which exist in the environment may account for differences in clinical manifestations between different localities.

Environmental sources of arsenic, aside from those listed above include some coal-fired power plants and nonferrous smelting operations. Natural sources include volcanoes and hot springs.

Although various analytical techniques are available for speciation of some arsenicals in air and water, others require better methods for accurate analysis at low concentrations. A system for interlaboratory crosschecking for analytic accuracy is needed. Several factors impinge on attempts to evaluate analytical data from human populations including media being examined (blood, urine, hair, nails), route and dose level etc. require methods capable of measuring total arsenic absorption from all routes of exposure.

There are very little data on kinetics and metabolism of arsenic and its compounds although it appears that much of a dose is excreted via the urine; some is degraded and some may be excreted without metabolic degradation and arsenic trioxide and pentoxide, in humans, appears to be excreted mainly in the methylated form.

There is some epidemiological evidence that high concentrations of arsenic in drinking water are associated with skin cancer. When the level was reduced by water treatment to 80 µg/liter, the incidence was reduced but still detectable. The existence of other cocarcinogens in these water supplies has not been extensively studied. If the time factors for the development of cancer are shown to be reasonable, then the current interim standard of 50 µg/liter may not provide an adequate margin of safety.

Recommendations for Research

1. Improvement and standardization of speciation techniques for analyses and application to various biological materials.

2. Interlaboratory crosschecking of the accuracy of the many methods using different matrices.

3. More accurate determination of the quantities of environmental arsenic, their sources and fate.

4. Studies about metabolism in man and animals; rates and mechanisms of methylation-demethylation in man, animals and ecosystems. Transfer of arsenic species across tissue barriers, absorption, distribution, and excretion.

5. Investigations about interactions of arsenic and other environmental factors which may account for difference in human clinical observations, and effects of diet, race, and climate.

6. Development of an animal model for carcinogenicity studies with particular reference to arsenic trioxide and pentoxide.

7. Studies about different responses to arsenic by individuals and species, particularly long term, low level exposure.

8. Further studies of the effect of arsenic on cellular mechanisms, as well as teratology and mutagenicity studies.

9. More uniform and improved methods for epidemiologic studies, coordinated by an international agency.

Selenium

Occurrence. Selenium is obtained industrially primarily in conjunction with electrolytic copper-refining. Selenium is used in manufacture of electronics equipment (rectifiers, photocells, and xerography), steel (for machinability and porosity control); pigments, glass (for decolorization and pigmentation), and ceramics (for colored glasses). It is used principally in elemental form and in such compounds as selenium dioxide, sodium selenite, sodium selenate, and iron selenate (Cooper, 1967).

Selenium has a profound effect on animals, and either a deficiency or an excess can result in adverse biologic responses. There is a relatively narrow margin of safety for many species and it is conceivable that an excess of selenium in drinking water can constitute a potential danger. Until recently, selenium was included in a list of carcinogic agents by the Food and Drug Administration (FDA), because of reports of animal research in the United States and Russia (Frost, 1960; Frost, 1972; Tscherkes *et al.*, 1963; Volgarev and Tscherkes, 1967). The evidence of carcinogenicity of selenium in these studies was tenuous and widely debated. The FDA now permits the addition of selenium to the feeds of turkeys, chickens, and swine and, because these feeds are mixed in commercial milling operations, the distribution of selenium may become wider than is now anticipated. Selenium can reach toxic concentrations in water from wells drilled through seleniferous shales rich in soluble selenium, and other sources of water contamination are known. Therefore, a better understanding of sources, distribution, metabolism, and health effects is required.

Water soluble selenium has been identified in soils (Olson *et al.*, 1942) and in some salt deposits (Byers *et al.*, 1938), and the presence of selenium in other geologic materials has also been documented (Beath, 1946). There is a wide variation in concentration of selenium, depending on geologic location. Thus, ground and surface waters may contain significant amounts of selenium, particularly in areas where there is an excess of selenium in rocks and soils; in other areas, there may be little (if any) detectable selenium in the water.

There is little in the literature to indicate that surface waters contain toxic amounts of selenium; in fact (Table V-13), it is likely that there is an insufficient amount of selenium in the water alone to provide the nutrient requirements of most animals (Cannon, 1974), but concentrations may vary in different places. A very extensive study by the Department of Health, Education, and Welfare involving analyses of 535 samples of water from major U.S. watersheds indicated that over a 4-y period only two samples contained selenium at more than 10 μg/liter of water, the U.S. drinking water standard (Lakin and Davidson, 1967). In another study, over a 2-y period, it was reported that there was a maximum of 10 and a mean of 4 μg/liter in 194 public finished water supplies (Taylor, 1963). In a study in Oregon, the majority of farm samples of water had less than 1 μg/liter (Hadjimarkos and Bonhorst, 1961). This study extended over a 2-y period and included samples from three counties. In Germany and in Australia, village water supplies have been reported to contain from less than 1 to 5.3 μg/liter (Oelschlager and Menke, 1969; Edmond, 1967). River water may contain high concentrations of selenium where irrigation drainage from seleniferous soils empties into it; value of 2,000 μg/liter have been reported (Williams and Byers, 1935).

TABLE V-13. Selenium in the Natural Environment

Material	Selenium Concentration, ppm	
	Average	Range
Igneous rock	0.05	-
Shale	0.60	-
Sandstone	0.05	-
Limestone	0.08	-
Coal (ash)	3.3	0.46-10.6
Phosphate rock	19.0	-
Soil	0.1-2.0	<0.4-1,200
Surface water	0.0002	0.0001-0.4
Forage grasses	0.36	<0.01-9.0
Forage legumes	0.2	0.075-0.7
Vegetables and fruits	0.05	0.01-0.20

(From Cannon, 1974)

The water from some springs and shallow wells contains selenium at more than 100 µg/liter (Byers *et al.*, 1938; Miller and Byers, 1935; Moretto and Diven, 1965). But, deep wells may contain only a few micrograms per liter. Water from some Wyoming wells contains enough selenium to be poisonous to man and livestock (Beath, 1962a), but these are in seleniferous areas. In another report from Wyoming, a high concentration of selenate in well water on an Indian reservation was associated with the loss of hair and nails in children (Beath, 1962).

Another source of selenium is the effluent from sewage plants which may contribute as much as 280 µg/liter in raw sewage, 45 µg/liter in primary effluent, and 5 µg/liter in secondary effluent (Baird *et al.*, 1972).

Of 818 samples obtained from interstate carrier systems, only one failed to meet the mandatory drinking water standard (Chemical Analysis of Interstate Carrier Water Supply Systems, USEPA, 1975).

It appears from a large number of reports that the selenium content of ocean water is very low. This is attributed primarily to the precipitation of selenite by oxides of iron and manganese (Goldschmidt and Strock, 1935; Ishibashi *et al.*, 1953; Strock, 1935).

In areas where the selenium content of the soil is high, the water in lakes may vary widely in selenium content (Abu-Erreish, 1967). Although the hypothesis that precipitation of selenium by various means results in low concentration in some cases, this has not been documented. Emerging data indicate that the microbial environment of lake-bottom sediment may influence selenium concentrations in the lake water (Chau *et al.*, 1976).

Available reports indicate little danger of toxicity from amounts of selenium in finished waters, but wells drilled through seleniferous shale containing soluble selenium may have concentrations of selenium high enough to be of concern. Furthermore, finished water for domestic consumption usually is not analyzed for selenium, and, although it appears that most waters have relatively low concentrations, in some cases the selenium may approach toxic concentrations. Recent studies by the U.S. Geological Survey reported that some farms near Denver, Colorado, had high selenium concentrations in their water supplies. Some of the water on a South Dakota Indian reservation contained as much as 210 µg/liter (U.S. Public Health Drinking Water Standards, 1972).

Water that is consumed by human populations rarely constitutes a significant source of selenium. Even with the very low concentrations of selenium found in rivers, there is a significant transport of the element into oceans. This has been estimated at as much as 8,000 tons of selenium per year discharged into the oceans from rivers (Bertine, 1971). For this reason, aside from quality, water is important in leaching and transporting the element under some conditions.

Selenium occurs naturally in the following oxidation states: selenide (-II), elemental selenium (0), selenite (+IV), and selenate (+VI). Recent published literature indicates that problems of environmental contamination are very likely minimized because nearly all organic selenium is in the -II oxidation state; this decomposes to elemental selenium, very little of which is absorbed (Klayman and Gunther, 1973; Okamoto and Gunther, 1972). Compounds of major concern to environmental toxicologists are selenite (+IV) and selenate (+VI). These are the forms that occur most often in water. The +II oxidation state has not been reported to occur naturally.

Selenate (+VI) is taken up from water or soil by plants and may reach toxic concentrations (Moxon et al., 1939). Selenite (+IV) salts are less soluble than the corresponding selenates. Of special interest is the very low solubility of the ferric selenites (Geering et al., 1968). It is also of considerable importance that selenite is rapidly reduced to elemental selenium under acid conditions by mild reducing agents, such as ascorbic acid. It is likely that selenite will either form insoluble compounds with ferric oxide or be reduced to insoluble elemental selenium, either of which would minimize the potential hazard in water.

Because elemental selenium is very insoluble and may be derived by high-temperature decomposition of some natural materials, a buffer of safety for water and other environmental sources is provided. For example, in the combustion of fossil fuels or organic materials, selenium dioxide, which is formed from elemental selenium, is reduced to elemental selenium by the sulfur dioxide that is formed during the combustion of these materials (Weiss et al., 1971). The amount of sulfur dioxide formed during such combustion is always greatly in excess of the amount required for the reduction of the selenium dioxide. For these reasons, elemental selenium appears to be a major inert form that provides a wide margin of safety by serving as a sink for selenium introduced into the environment.

Selenide (-II) which occurs as hydrogen selenide is a strong acid with toxic fumes. Hydrogen selenide, however, decomposes rapidly in air to form elemental selenium and water, thus eliminating the hazard from this compound for most people, except those involved in industrial installations. It appears that a large amount of insoluble selenide and possibly elemental selenium pass through most animals without appreciable absorption; this is particularly so in ruminants (Peterson and Spedding, 1963).

The geochemical behavior of selenium in water resembles that of sulfur. Both are volatile and are emitted as gases during the natural course of volcanic eruption, during the smelting of sulfide ores, and in the burning of coal. It is only where the water-soluble selenate ion occurs in soils that plants can take up significant amounts of it. For this reason, cretaceous shales of a relatively low selenium content produce toxic vegetation, whereas soils that have a much higher selenium content (e.g., in Hawaii and Puerto Rico) do not produce toxic amounts in plants, because the selenium occurs as selenite bound to ferric oxide (Lakin, 1972; Cannon, 1974).

In the +IV state, selenium occurs as organic selenite. Soluble selenites are highly toxic. Selenite binds easily to iron and aluminum, with which it forms stable absorption complexes.

Alkaline water conditions favor the formation of the +VI form, selenate. Selenates are quite soluble, highly toxic, not tightly complexed by sesquioxides, easily leached from soils, and available to plants.

In general, one would expect to find higher amounts of selenium in foods and water in areas that have been designated as seleniferous. The data in a number of reports suggest that feeds that are highest in protein usually are highest in selenium content (Scott and Thompson, 1971). It may be concluded, however, that the selenium concentration in plants depends largely on the concentration and availability of selenium in the soil where the plants are grown. For example, in South Dakota whole milk may contain 1.2 ppm, whole eggs as much as 10 ppm, and vegetables (string beans, lettuce, turnip leaves, and cabbage) from 2-100 ppm; the concentrations in the milk and eggs probably reflect that in the feed. A number of investigators have found samples of wheat and wheat products that contain selenium at 1-4 ppm (Lakin and Byers, 1941; Robinson, 1936). Selenium concentrations in the gluten fractions are usually 4-5 times greater than that in the whole wheat.

Foods from nonseleniferous areas contribute very little to the overall dietary intake of selenium. Eggs and milk, fish, various types of meat, poultry, coffee, and tea all vary somewhat in their selenium content, but in general contribute minimally to the dietary intake. Table V-14 lists the selenium content of staple foods of the American diet (Morris and Levander, 1970).

There are occasional cases of industrial exposure to selenium when it is used in relatively high concentrations; the most notable is in copper refining, where selenium is a byproduct. Potential indurtrial sources of exposure to selenium have been reviewed (Davis, 1972; Dudley, 1938). Table V-15 (derived from Davis, 1972) provides estimates of selenium emission factors.

Selenium is not normally used in agriculture, so it is unlikely that agriculture itself will contribute a significant amount to the water supplies. However, now that selenium is being added to the feeds of turkeys, chickens, and swine, some slight increase in soil concentrations may take place; if this occurs, runoff water might increase the selenium content of lakes and streams to a small degree.

TABLE V-18
Selenium Content of Some Foods in the American Diet*

Food	Average Selenium Content,** ppm (wet wt.)
Vegetables, canned and fresh	0.010 (0.004-0.039)
Fresh garlic	0.249
Mushrooms, canned and fresh	0.118
Fruits, canned and fresh	0.006 (<0.002-0.013)
Cereal products	0.38 (0.026-0.665)
Egg white	0.051
Egg yolk	0.183
Brown sugar	0.11
White sugar	0.003
Cheeses	0.092 (0.052-0.105)
Table cream	0.006
Whole milk	0.012
Meat (excluding kidney)	0.224 (0.116-0.432)
Seafood	0.532 (0.337-0.658)

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*Derived from Morris and Levander (1970).

**Figures in parentheses are ranges.

TABLE V-15
Estimated Selenium Emission Factors*

Source	Estimated Selenium Emission Factors*
Mining and milling:	
Copper	0.015 lb/thousand tons of ore mined
Lead	0.047 Do.
Zinc	0.032 Do.
Phosphate (western)	0.350 Do.
Uranium	0.350 Do.
Smelting and refining:	
Copper	0.25 lb/ton of copper produced
Lead	0.05 lb/ton of lead produced
Zinc	0.04 lb/ton of zinc produced
Selenium refining:	
Primary (from copper byproducts)	277 lb/ton of selenium recovered
Secondary	100 Do.
End product manufacturing:	
Glass and ceramics	700 lb/ton of selenium consumed
Electronics and electric	2 Do.
Duplicating	2 Do.
Pigments	15 Do.
Iron and steel alloys	1,000 Do.
Other	10 Do.
Other:	
Coal	2.90 lb/1,000 tons of coal burned
Oil	0.21 lb/1,000 barrels of oil burned
Incineration	0.02 lb/1,000 tons of refuse burned

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*Derived from Davis (1972).

Metabolism. The absorption, distribution, biotransformation, and excretion of selenium in microorganisms, plants, animals, and humans have recently been reviewed in detail (NAS, 1976). The present discussion is focused on selenium metabolism in animals and humans.

Absorption. Both inorganic and organic forms of selenium can be readily absorbed from the gastrointestinal tract. Although little is known about the uptake of ingested selenates, selenites are passively absorbed from mammalian intestine (McConnell and Cho, 1965; Brown *et al.*, 1972). Selenite is absorbed more rapidly by monogastric animals than in ruminant animals, perhaps owing to bacterial reduction of selenite to elemental selenium or other insoluble forms in the ruminant gastrointestinal tract (Wright and Bell, 1966).

Selenocystine is passively absorbed from the intestine, but in the hamster selenomethionine can be absorbed against a concentration gradient; the active absorption of selenomethionine is inhibited by low concentrations of S-methionine (Spencer and Blau, 1962; McConnel and Cho, 1965). Absorption of sodium selenite through rat skin has been reported (Dutkiewicz *et al.*, 1971).

Distribution. In the dog, as selenate is absorbed it binds to plasma albumin, gradually shifts to globulins, and finally becomes associated with red cells (McConnel, 1941; McConnel *et al.*, 1960). The uptake of selenium by red cells varies inversely with dietary concentration (Wright and Bell, 1963), and this relationship has been proposed for several groups of animals and humans (Weswig *et al.*, 1966; Burk *et al.*, 1967; Lopez *et al.*, 1968).

Selenite and selenate, after single subacute administration to rats (McConnell, 1941) and mice (Heinrich and Kelsey, 1955) are distributed largely to the liver, kidneys, muscle mass, gastrointestinal tract, and blood. In rats, selenite distribution to the liver appears to be unaffected by the concentration of selenium in the maintenance diet (0.04-5.04 ppm), but a greater percentage of administered selenite goes the kidney, blood, and muscles in rats on low-selenium diets, compared with animals maintained at higher selenium concentrations (Hopkins *et al.*, 1966). With chronic administration, selenium is also distributed to the testes (Broyer *et al.*, 1966; Burk *et al.*, 1972).

Selenite distribution within the liver cell has been determined. More than half is in the cytosol and, depending on dose and diet, the rest is variably distributed in other fractions; injected selenite is found more in the mitochondria than in micromomes or nuclei (McConnel and Roth, 1962), whereas, in rats fed a selenium deficient diet, nuclei and microsomes have about equal amounts of selenium and much more than mitochondria (Brown and Burk, 1973). Selenium in the liver turns over rapidly.

Selenoamino acids and inorganic selenium are distributed similarly,, except that selenomethionine and selenocystine accumulate in the pancreas to a greater extent than selenite (Jacobsson, 1966). When mice are fed alfalfa grown on selenium -75 selenious acid, the radiolabel is found more in kidneys than in liver or pancreas and to a much lesser extent in lungs, heart, spleen, skin, and brain (Jones and Godwin, 1962, 1963).

Studies on selenium distribution in pregnant ewes have demonstrated a placental barrier. The maternal fetal plasma ratio of selenium concentrations sheep was 12:1 for a single fetus and 22:1 in the case of double fetuses (Wright and Bell, 1966). Selenium transfer across the placenta was slow but continuous.

In many instances, the distribution of selenium to tissues and cells depends on the animal's nutritional status regarding selenium; the time after administration is also important, in that selenium distributions shift with time (Wright, 1967).

Excretion. The principal route of excretion of selenium is via the urine. Approximately 40% of the selenate administered to rats is excreted in the urine in the first 24 h, and the rate of excretion is much lower after that (McConnell, 1941). The dietary concentration of selenium has a pronounced effect on excretion; rats maintained on low-selenium diets (0.004 ppm) excreted 6% of a dose of radioselenium in the urine in 10 days, and animals on high-selenium diets (1 ppm) excreted 67% of an equal dose of radioselenium in the same time (Burk *et al.*, 1972). Whether this represents an ability to conserve selenium when intake is low or an increased ability to excrete selenium when intake is high is not clear.

Fecal excretion of selenium by rats is small in most situations other than poisoning (Burk *et al.*, 1972). In swine, fecal excretion of selenium was about 5 times greater when sodium selenite was administered orally than when it was administered intravenously, although the total excretion was the same, regardless of route of administration (Wright and Bell, 1966). Fecal excretion of selenium by sheep was approximately the same as in swine after intravenous injection of sodium selenite ,but increased 13 fold after oral administration. Swine absorb selenium better than sheep, and the greater fecal excretion by sheep may be due to bacterial reduction of selenite to insoluble elemental selenium.

Pulmonary excretion of selenium is important only in subacute poisoning and depends on the dietary concentrations of protein, methionine, and selenium (Olson *et al.*, 1963).

Storage. Selenium binds to cystine-rich keratin and consequently is found in hair and nails (McConnell and Kreamer, 1960). When wool is chemically reduced and treated with selenium dioxide, the selenium forms a selenodithio bridge between two cysteine units (R-S-Se-S-R) (Holker and Speakman, 1958).

Radioselenium injected into dogs was retained in hair for as long as 318 days (McConnell and Kreamer, 1960). The effect of diet on the long-term rate of loss of selenium has been studied in rats; increasing the dietary selenium content decreases selenium retention (Burk *et al.*, 1972, 1973). Cattle, sheep, and swine fed inorganic selenium for several weeks take 10-20 w to return to baseline tissue selenium concentrations when put on depletion diets; if the animals are fed organic forms of selenium, it takes even longer for tissue concentrations to return to baseline (Kuttler *et al.*, 1961; Hidiroglou *et al.*, 1971; Ku *et al.*, 1972).

Biotransformation. Little is known about the biochemistry of selenium in mammalian systems. At concentrations required nutritionally, selenium is incorporated into specific functional proteins; at higher concentrations, it is incorporated into molecules normally served by sulfur. Selenium analogs are often less stable than sulfur compounds, and this lability may be the basis of toxicity. Selenium biochemistry has been the subject of recent reviews (Stadtman, 1974; NAS, 1976).

By the mechanism used for sulfate ion, microorganisms are capable of activating selenate with adenosine triphosphate (Wilson and Bandurski, 1956), but it is not clear that appreciable amounts of activated selenate (APSe) are reduced to selenite via 3'-phosphoadenosine-5'-phosphoselenate (PAPSe), which would be directly analogous to the recognized reduction of activated sulfate (APS) to sulfite by phosphoadenosine phosphosulfate (PAPS). In animals, PAPS is important in the formation of sulfate esters in the detoxication of foreign compounds and the metabolism of steroids and other indigenous compounds (Lipmann, 1958); the activity of PAPSe, if formed, in formation of selenate esters is not known. Although selenate and selenite ions are absorbed and incorporated into organic molecules as selenide, it is not fully known how the reduction of selenium is accomplished (Stadtman, 1974).

Selenite is methylated by mammalian tissues in an apparent detoxication process. Mouse liver and kidneys use Sadenosylmethionine and reduced glutathione to form dimethylselenide from selenite (Ganther, 1966); the lungs are also active in the methylation, but muscle, spleen, and heart have little activity. Dimethylselenide is less toxic than sodium selenite (McConnell and Portman, 1952a). Sodium selenate is also reduced and converted to dimethylselenide in rats (McConnell and Portman, 1952b).

Selenite and selenate are metabolized to trimethylselenonium ion, $(CH_3)_3Se^+$ which is the principal excretory product of selenium in urine (30-50% of the urinary selenium) (Byard, 1969; Palmer *et al.*, 1969, 1970). Again, trimethylselenonium ion is less toxic than selenite or selenate ion (Obermeyer *et al.*, 1971). Although these methylated products are less toxic than the parent selenium compounds, they are involved by unknown mechanisms in synergistic toxicity; dimethylselenide and mercury toxicities are synergistic (Parizek *et al.*, 1971), as are those of trimethylselenonium ion and arsenic (Obermeyer *et al.* (1971).

In mammalian systems, inorganic selenium usually is not incorporated into amino acids (Cummins and Martin, 1967), although there is some evidence of the incorporation of selenium from sodium selenite into a rabbit protein (Godwin and Fuss, 1972). The matter is confusing, because inorganic selenium can be reduced to complex with disulfides to give selenodisulfides (R-S-Se-S-R), as is the case with two molecules of cysteine (Painter, 1941; Ganther, 1968) or reduced glutathione (Ganther, 1971).

Selenium appears to serve as an essential element in some oxidation-reduction processes in mammals. Sheep skeletal muscles contain a small (mol. wt., 10,000) selenoprotein that has a heme group. Although the selenium appears to be an integral part of the protein, its position and function in the protein are not known (Pedersen *et al.*, 1973).

A second selenoprotein is known: glutathione peroxidase, an enzyme, catalyses the reduction of hydrogen peroxide. The activity of glutathione peroxidase in red cells of selenium-deficient animals is low, but may be restored specifically by selenium administration (Rotruck *et al.*, 1973). The enzyme has a molecular weight of 84,000 and is composed of four subunits of molecular weight 21,000 each; each subunit contains one atom of selenium (Flohe *et al.*, 1973).

Health Effects. Although the essentiality of selenium as a nutrient for domestic and laboratory animals was established only fairly recently, its toxicity in animals has been known for more than a century. As a toxic element, selenium may produce a variety of clinical and toxicological syndromes, depending on the animal species, the dose, and the duration of exposure.

Toxic Effects in Humans. Reviews of selenium toxicity in humans include papers by Cooper (1967), Cerwenka and Cooper (1961), and Amor and Pringle (1945) and a monograph by the NRC Subcommittee on Selenium (NAS, 1976).

Elemental selenium is relatively nontoxic, but some compounds such as soluble salts of selenium dioxide, selenium trioxide, and some halogen compounds are toxic in humans with hydrogen selenide one of the most toxic and irritating selenium compounds. The toxic vapors and soluble salts are readily absorbed by the tissues of the lungs and alimentary canal and perhaps by the skin.

Exposure of humans to selenium in most industrial situations is through the skin and lungs by exposure to dust or fumes. Selenium fumes in sufficient concentration can produce an acute respiratory distress syndrome in exposed humans. Most acute exposures to selenium and its compounds result in such symptoms as irritation of eyes and mucous membranes, sneezing, coughing, dizziness, dyspnea, dermatitis, headaches, pulmonary edema, nausea and garlic breath odor. Prolonged exposure can result in death (Clinton, 1947; Glover, 1954, 1970; Middleton, 1947; Dudley, 1938; Buchan, 1947; Dudley and Miller, 1941; Carter, 1966).

Chronic exposure of humans to selenium by ingestion or via the lungs (by inhalation of dusts and fumes) produced a set of signs and symptoms that included depression, nervousness, occasional dermatitis, gastrointestinal disturbance, giddiness, and garlic odor of the breath and sweat (Cooper, 1967; Lemley and Merryman, 1941). Amor and Pringle (1945) and Glover (1970) considered the presence of a garlic breath odor to be the earliest and most characteristic sign of selenium absorption. However, the garlic odor is not specific for selenium, inasmuch as it is observed after absorpion of tellurium. Garlic odor after selenium absorption is apparently due to formation of dimethyl selenium (Glover, 1970; Carter, 1966). Motley *et al.* (1937) suggested that the elimination of dimethyl selenium in the breath may give rise to sore throats and pneumonitis.

Selenium has been implicated as having an influence on the incidence of dental caries. Epidemiologic studies of children indicated that the small amounts of selenium present in foodstuffs in some regions were significant in increasing the incidence of dental caries if consumed during the period of the development of the teeth (Hadjimarkos and Bonhorst, 1958; Tank and Storvick, 1960). Studies in rats indicate a cariogenic role for selenium; Buttner (1963) reported that the addition of selenium at 2.3 and 4.6 ppm as sodium selenite to the drinking water of pregnant rats and to their offspring increased the incidence of caries in proportion to the amount of selenium present and also reduced the number of young born.

Nagai (1959) described signs of poisoning in Japan among workers in the manufacture of selenium rectifiers. Long employment was followed by hypochromic anemia and leukopenia. Female workers had irregular menses or menostasis.

Smith and Westfall (1937), in a detailed study of correlate symptomatology with selenium excretion and selenium intake in the diet in humans in a highly seleniferous area, found that the most frequent symptoms were gastrointestinal disturbances, bad teeth, icteroid discoloration of the skin, and sallow and pallid skin color in younger people. None of the symptoms were regarded as specific effects of selenium intake, and it was not certain that any resulted directly from continual ingestion of selenium.

Therapeutic uses. Selenium sulfide combined with bentonite and mixed with detergent is marketed under the trade name Selsun as a shampoo. The combination of selenium sulfide and detergent has been used in the treatment of seborrheic dermatitis and of tinea versicolor. The literature dealing with the therapeutic effects of selenium in seborrheic dermatitis was reviewed by Matson (1956). Cohen (1954) and Fritz (1955) reported that the selenium sulfide suspension was effective in the treatment of granulated eyelids (blepharitis marginalis). Eisenberg (1955) and Grover (1956) reported unfavorable reactions such as loss of hair and local skin irritation after the use of Selsun as a therapeutic agent. Albright and Hitch (1966), Giordano (1963), Levan (1957), and Robinson and Yaffee (1957) used selenium sulfide in the treatment of tinea versicolor. How useful the treatment with selenium has been has not been established by adequate follow up studies. Ransone *et al.* (1961) described a case of systemic selenium toxicity in a woman with open scalp lesions who had used the shampoo two or three times a week for 8 months.

Diagnostic Uses. Blau and Manske (1961) found by the use of (^{75}Se) selenomethionine in dogs that the compound had sufficient specificity for localization in the pancreas that it could be used for visualization of the pancreas by isotope scanning methods. There are no known precautions or contraindications to the use of (^{75}Se)selenomethionine for scanning of human pancreas as the total selenium required is approximately 50 μg. (Rosenfeld and Beath, 1964). Herrera *et al.* (1965) reported that (^{75}Se)selenonmethionine given intravenously for scanning of the pancreas was incorporated sufficiently into cells of lymphomas to allow detection of these tumors.

Potchen (1963), DiGiulio and Beirwaltes (1964), and Haynie *et al.* (1964) have reported that the localization of [^{75}Se]selenomethionine was sufficiently higher in the parathyroid gland than in the thyroid gland and other surrounding tissues to make it usable for localization of parathyroid gland adenoma.

Garrow and Douglas (1968) suggested the use of (^{75}Se)selenomethionine for the measurement of placental competence. Douglas (1969) assessed the possible dangers and found that the test cannot be considered dangerous, because the radiation dose is too low to be considered harmful to a fetus. Lee and Garrow (1970) found no adverse reactions after its use in 467 patients.

Toxic Effects on Animals. Domestic Animals. Selenium toxicity in domestic animals has been the subject of several reviews (Anderson *et al.*, 1961; Harr and Muth, 1972; Rosenfeld and Beath, 1964; Muth and Binns, 1964). Toxicity syndromes may be acute or chronic. The acute form results from the ingestion or injection of large quantities of selenium. Chronic selenosis is due to consumption of small amounts of selenium compounds over w or months. In some studies in domestic animals with inorganic forms of selenium, chronic administration eventually resulted in an acute syndrome followed within a short period by death.

Shortridge *et al.* (1971) described an accidental occurrence of acute selenium poisoning in calves. The animals were to receive prophylactic subcutaneous injections of a sodium selenite solution; owing to an error in the preparation of the solution, they received 100 mg of selenium (approximately 0.5 mg/kg of body weight), instead of the intended 12 mg. The calves were depressed and salivating and had respiratory distress within 2 h of injection. Within 5 weeks, 67% had died. Dyspnea was the most noticeable clinical sign in the calves that died.

A few reports detail the acute toxic signs and lesions in sheep caused by selenium poisoning, including papers by Caravaggi *et al.* (1970); Gabbedy and Dickson (1969); Morrow (1968), and Lambourne and Mason (1969). In the accidental poisoning cases described by Morrow (1968), young lambs were given 10 mg of sodium selenite orally. Seven died within 10-16 h; eight others developed diarrhea, but recovered; and five were unaffected.

Herigstad *et al.* (1973) studied the toxicosis produced by sodium selenite (inorganic) and selenomethionine (organic) in young swine. Concentration in the ration varied from 0.1-600 ppm. At higher doses, survival varied from 1-10 days. A dose of

3 mg of selenium produced fatal selenium toxicosis within 2.5-14 h.

Laboratory Animals. Franke and Potter (1936) found that the minimal fatal dose the (smallest dose that killed 75% or more of the animals in 48 h) of selenium as sodium selenite injected intraperitoneally was 7.3 mg/kg of body weight. Smith et al. (1937) reported the intravenous LD_{50} of selenium as both selenite and selenate to be 3.0 mg/kg of body weight.

Hopkins et al. (1966) reported poor growth in weanling rats fed various semipurified diets containing selenium at 5 ppm as selenite for 2 weeks when the ration was composed of natural feedstuffs.

Halverson et al. (1966) fed selenium as sodium selenite and as seleniferous wheat to young rats at concentrations of 1.6, 3.2, 4.8, 6.4, 8.0, 9.6, and 11.2 ppm in a wheat diet. Growth depression occurred when the diet contained 6.4 ppm selenium or more. Mortality occurred after the fourth week of feeding at selenium concentrations of 8.0 ppm or more. A concentration of 8.0 ppm resulted in enlargement of the pancreas, reduction of hemoglobin content, and increased serum bilirubin.

Palmer and Olson (1974) administered several concentrations of sodium selenite or sodium selenate in the drinking water to weanling rats. Selenium at concentrations of 2 or 3 ppm produced only a small reduction in weight gain and no mortality. Rats given water containing either form of selenium at 6 or 9 ppm had increased mortality. Smith et al. (1937) found that the toxicity of the selenite and selenate ions was similar when given intravenously and intraperitoneal. Franke and Moxon (1936) reported that rats fed seleniferous grains reduced their feed intake, lost weight or gained weight slowly, developed a hunched back, and had yellow staining of fur about the genitals. Necropsy findings included general visceral congestion, anemia, and cirrhosis of the liver.

Morss and Olcott (1967) administered selenium to rats orally at 10-15 mg/kg of body weight. The rats lost weight, and many developed diarrhea and had bleeding from the nose, lacrimation, and depression. The LD_{50} was approximately 12.5 mg/kg.

Halverson et al. (1970) reported that the anemia produced in rats by feeding of sodium selenite at 5-15 ppm was due to hemolysis. The synthesis of new red cells appeared unaffected.

Campo and Biein (1971) found histologic changes of the epiphyseal plate in rats given selenium at 4-58 mg/kg of body weight intraperitoneally as sodium selenate for 13 days. Changes included blurring of the cellular and lacunar outlines, decrease in basophilia, disruption of cell columnation, and increase in widths of zones of proliferating and maturing chondrocytes.

McConnell and Portman (1952a) found that dimethyl selenide had a low degree of toxicity in mice; LD_{50} was 1.3 g/kg by intraperitoneal injection. A state of hyperpnea lasted for 2-3 h, and the breath had a garlic odor. Convulsions were followed by death within a few hours of administration in most mice, but a few lived for 36 h. Mautner and Jaffee (1958) reported that the LD_{50} of 6-selenopurine by intraperitoneal injection was 160 ± 17 mg/kg of body weight.

Hadjimarkos (1970) gave male hamsters selenium as sodium selenite in their drinking water at 6, 9, and 12 ppm. Signs of toxicity were not observed at 6 ppm, but concentrations of 9 and 12 ppm caused a reduction in weight gain. These groups also consumed less water--45% less than controls.

Smith et al. (1937) studied selenium toxicity in cats and reported that the minimal lethal dose of selenium as sodium selenate or selenite was about 1.5-3.0 mg/kg of body weight, regardless of the route of administration; subcutaneous, intraperitoneal, and intravenous routes were investigated.

Rhian and Moxon (1943) compared the toxic effects of seleniferous grain and inorganic selenium as sodium selenite in dogs. Manifestations of selenium intoxication were similar to those in other laboratory animals. Signs appeared when the diet contained selenium at 7.2 ppm as seleniferous grain and at 10 ppm as selenite. A selenium concentration of 20 ppm caused anorexia and death within a short time.

Schroeder and Mitchener (1971) administered selenate and selenite to rats in the drinking water at 2 ppm for a year and then at 3 ppm for another year. Selenate at these intakes was not toxic, with respect to growth, survival, and longevity. Rosenfeld and Beath (1947), Smith et al. (1937), and Smith (1941) described the signs and lesions in rats undergoing chronic selenium poisoning: marked loss of body weight to cachexia, often accompanied by ascites and hydrothorax; hunched backs; coarse, disheveled pelage; and anemia.

Schroeder and Mitchener (1972) gave selenium as sodium selenite and sodium selenate to mice in the drinking water at 3 ppm for life. The two forms of selenium did not produce signs of toxicity with respect to growth, survival, or longevity in males; longevity in females was decreased. The incidence of spontaneous tumors was not affected. Schroeder and Mitchener (1971b) reported that selenate fed to pregnant mice in successive generations was very toxic at doses tolerated in weanling and nonpregnant adult mice. The results were death, failure to breed, and production of stunted mice.

Animal experiments indicate that the young are more susceptible than adults of the same species. Franke and Potter (1936) reported that the tolerance of rats to seleniferous diets increases markedly between the ages of 21 and 42 days.

Carcinogenicity. Nelson *et al.* (1943) reported the induction of cirrhosis and tumors in the livers of male Osborne-Mendel rats fed either seleniferous grain or a solution of ammonium potassium sulfide and ammonium potassium selenide. The protein concentration of the diets was 12% and selenium concentrations were 5, 7, and 10 ppm. Of 53 rats surviving for 18 months, 11 developed tumors in cirrhotic livers and the other 42 contained focal hyperplasias. No metastatic growths were observed, and no tumors occurred in livers of rats not surviving for 18 months. The hepatic tumors were composed of regular or irregular cords of large hepatocytes without prominent cellular atypism.

Tscherkes and co-workers (1963) fed male rats a 12% protein diet containing selenium at 0.43 mg/100 g of diet. Of 23 rats surviving for 18 months, 10 had tumors: three had malignant hepatic tumors, and two of these had lung metastases; four had sarcomas; and three had hepatic adenomas. Three of 40 rats fed selenium at 0.86 mg/100 g of diet had sarcomas in the mediastinal and retroperitoneal lymph nodes. In a later study by Volgarev and Tscherkes (1967), the incidence of tumors designated "carcinoma" was reduced when protein in the diet was increased from 12-30%. The tumor incidence was 8.5% in 200 rats. The incidence of tumors was 35% in rats fed a diet with selenium at 0.43 mg/100 g and 12% casein; 8.5% in rats fed selenium at 0.86 mg/100 g and 30% casein; and 0% in a group fed selenium at 0.43 mg/100 g, 12% casein, and dietary additives.

Harr *et al.* (1967) fed female Wistar rats commercial and semipurified diets containing selenium at several concentrations, including 2 ppm with 12% casein; 2, 6 and 9 ppm with 22% casein; and 4, 6, and 8 ppm with methionine and 12% casein. The hepatic lesions were designated acute and chronic hepatitis and focal hyperplasia with marked cellular atypism. None of the rats had hepatic cirrhosis. Hyperplasias were most numerous in rats fed a control diet for 50-250 days after an 84-days period of selenium feeding and rats fed selenium and control diets in alternate weeks. Fewer toxic and hyperplastic lesions were found in rats fed selenium-supplemented commercial diets than in rats fed the purified diets with added selenium. They concluded that selenium as selenite and selenate was not carcinogenic in the rat.

Schroeder and Mitchener (1971) reported tumor incidences of 30% in older male rats fed selenite at 3 ppm and 75% in older female rats. Of the tumors, 14% of those in the males and 52% of those in the females were considered malignant. The rate of metastatic lesions was high. In a later study by Schroeder and Mitchener (1972), in which rats were given selenium at 2 ppm as sodium selenite and sodium selenate for a year and then changed to selenium at 3 ppm, the incidence of tumors in the selenate group was 62.5% (control, 31%) and the incidence of malignant tumors was 42% (control, 17%).

The "positive" studies do not establish selenium as a carcinogen in that there were several deficiencies in the experimental design and the interpretation of the lesions:

1. It is possible and probable that some of the "low grade carcinomas" described by Nelson and colleagues (1943) were degenerative lesions in the cirrhotic livers, rather than tumors.
2. It seems likely that acetaminophenyl selenium dehydroxide has an inherent goitrogenic activity apart from its selenium content, and there are no other reports that selenium is tumorigenic for the thyroid.
3. The low incidence of "spontaneous" tumors occurs in rats not receiving selenium and variations observed by several investigators can be due in part to age, sex, strain, diet, and contamination of diets with other carcinogens.
4. The reports of Schroeder and Mitchener (1971a) have not been confirmed or sufficiently documented.
5. Epidemiologic and demographic studies do not suggest that selenium is carcinogenic. Some regions of the world, including the north central and Rocky Mountain regions of the United States are geologically rich in selenium. The incidence of cancer in humans is lower in these regions than in nonseleniferous regions (Shamberger, 1970).
6. Selenium is not carcinogenic in the mouse (Schroeder and Mitchener, 1972a).

Mutagenicity. No reports of mutagenicity by selenium compounds were found.

Teratogenicity. The chick embryo is extremely sensitive to low concentrations of selenium. Concentrations in feeds too low to produce signs of poisoning in adult poultry and other farm animals have reduced hatchability and produced deformities in chicks. Poor hatchability of eggs due to deformities in the chicks was found by Franke and Tully (1935) to occur in areas where toxic feedstuffs were produced.

Holmberg and Ferm (1969) found that selenium as sodium selenite at 2 mg/kg of body weight was not teratogenic in hamsters when injected intravenously on the eighth day of pregnancy.

Reproduction. Franke and Potter (1935) fed seleniferous-wheat diets to rats at ages of 21-186 days. Rats that survived the toxic diets for relatively long periods had subnormal growth and reduced reproductive capacity. Matings between animals that were fed the selenium diets were infertile. Matings in which one animal was fed the normal diet and the other a toxic diet were sometimes fertile, but poisoned females were unable to raise their young. Rosenfeld and Beath (1954) provided pregnant rats with water containing selenium at 1.5, 2.5, and 7.5 ppm as potassium selenate. Normal litters were obtained from females given the seleniferous water at 1.5 and 2.5 ppm. The second generation of selenium-dosed rats had normal litters, but the number of weaned pups was decreased by about 50%. In the group of females given the water with selenium at 7.5 ppm, fertility was reduced, the number of survivors was decreased, and growth of the young was reduced. When water containing 7.5 ppm selenium was provided on days 5-8 before parturition, the rats had normal litters, but the number of pups weaned was reduced with the continued intake of selenium. By mating of normal with selenium exposed animals, it was determined that failure of reproduction in the rats was due to the effect of selenium on the female. Schroeder and Mitchener (1971b) gave mice selenium in the drinking water from weaning through several generations. Normal litters were produced until the third generation, which contained fewer and smaller litters with runts. Failure to breed and excessive deaths before weaning were also observed.

Interactions. Interrelationships of selenium toxicity with arsenic, mercury, cadmium, silver, and thallium have been described (Diplock, 1976).

Moxon (1938) established that acute and chronic toxicity produced by feeding of seleniferous grains containing selenium at 15 ppm could be alleviated or prevented by administration of arsenic at 5 ppm as sodium arsenate in the drinking water. Ganther and Baumann (1962) used subacute dosages of arsenic and selenium and found that excretion of selenium into the gastrointestinal tract was stimulated by arsenic. Levander and Baumann (1966) demonstrated that bile was the route of excretion for selenium in arsenic-treated animals. A tenfold increase in the amount of selenium excreted into the bile of rats prepared with acute biliary fistulas was found. Levander (1972) suggested that arsenic protection against selenium toxicity may be mediated by combination of arsenic with selenium in the liver through reaction to selenol compounds to form a detoxication conjugate readily passed into the bile.

Levander and Argrett (1969) described the effect of mercuric salts on the metabolism of selenium. Mercury increased the retention of selenium in the blood, kidneys, and spleen. Parizek *et al.* (1974) reported that selenium compounds protected against the toxicity of mercury. The renal and intestinal lesions produced by mercuric chloride at 20 µmol/kg of body weight were abolished by the same dose of selenium as sodium selenite when given 1 h after mercuric chloride. Rats given lethal doses of mercuric chloride with selenium survived, and there were few gross lesions. The excretion of mercury was decreased by selenium. Parizek *et al.* (1971) found that the transport of mercury across the placenta in pregnant rats was decreased by selenium, and less mercury was secreted into the milk. The bioavailability of selenium was much lower in the rats treated with mercury. Ganther *et al.* (1972) reported that survival of Japanese quail given mercury at 20 ppm as methylmercury in diets containing 17% tuna was considerably longer than survival of quail exposed to the same concentration of methylmercury in a corn-soya diet. A striking correlation was found between the concentrations of selenium and mercury in the batches of tuna; batches that had little selenium contained low concentrations of mercury, and, when the concentration of mercury was high, the selenium concentration was also high (selenium:mercury, 2.91:2.97 ppm). The selenium in the tuna lowered the toxicity in the quail of the methylmercury added at 20 ppm. In an experiment with rats fed a basal diet containing 20% casein with and without the addition of selenium at 0.5 ppm as sodium selenite, it was found that mercury at 10 ppm as methylmercury produced 100% mortality after 6 w of feeding, but selenium was completely effective in preventing mortality.

Parizek and Zahor (1956) reported that the administration of cadmium at subtoxic concentrations by subcutaneous injection produced necrosis of the testes of rats. Kar *et al.* (1960) found that the cadmium-induced lesions in the testes could be prevented by the administration of selenium. Mason and Young (1967) reported that the testicular injury produced by single subcutaneous injections of 0.45 mg of cadmium chloride in rats was protected against by half-equimolar selenium dioxide injected at the same time as cadmium. Protection was also provided by daily subcutaneous injections of half-equimolar selenium dioxide given over 6 successive days before cadmium. Parizek *et al.* (1968) and Gunn *et al.* (1968) found that mortality of rats given lethal
doses of cadmium was much reduced by administration of selenium. Holmberg and Ferm (1969) found that the teratogenicity of cadmium was considerably reduced by selenium. Kar *et al.* (1959) and Parizek *et al.* (1968) found that cadmium would selectively damage the nonovulating ovary in the rat and that this damage was prevented by administration of selenium. Parizek *et al.* (1968)

and Parizek (1964) found that cadmium produced necrosis and destruction of the placenta and that these changes could be prevented by the administration of selenium. Similarly, the "toxemia of pregnancy" induced by cadmium could be prevented by selenium (Parizek, 1965).

Diplock *et al.* (1967) and Grasso *et al.* (1969) reported that 0.15% dietary silver acetate produced toxicity in rats and chicks deficient in vitamin E. When the diets were adequate in vitamin E and selenium, the silver was not toxic. The removal of the vitamin E resulted in 100% mortality within 49-64 days. The addition of selenium to the diet at 1.0 ppm produced a protection of 55% against toxic effects of silver. Grasso *et al.* (1969) studied the lesions produced in the liver by silver and the lesions caused by dietary deprivation of vitamin E and selenium. The lesions were similar.

Hollo and Zlatarov (1960) reported that mortality induced by thallium poisoning could be prevented by the parenteral administration of selenate. Rusiecki and Brzezinski (1966) found that oral administration of selenate prevented the toxicity of thallium and that the content of thallium in liver, kindeys, and bones was increased by the selenate. Levander and Argrett (1969) observed that the subcutaneous injection of thallium acetate increased the retention of selenium in the liver and kidneys and decreased the pulmonary and urinary excretion of selenium.

Levander and Morris (1970) used a peanut-meal diet and found that neither methionine nor vitamin E alone gave much protection against hepatic damage produced by excessive selenium. Combinations of methionine and vitamin E were effective, and the degree of protection was approximately proportional to the concentration of vitamin E added to the diet. Selenium concentrations of the liver and kidneys from rats fed the diets supplemented with methionine and vitamin E were less than those of the same organs from rats fed either methionine or vitamin E alone or no supplement. The results were compatible with the hypothesis that methionine detoxifies selenium by forming methylated derivatives of the element that are eliminated via the breath and the urine. Vitamin E and some fat-soluble antioxidants increase the availability of the methyl group of methionine for this process.

Moxon and DuBois (1939) reported that the combined administration of fluoride and selenium to rats increased the toxicity of selenium. They added fluoride at 5 ppm to the drinking water of young rats fed a diet containing selenium at 11 ppm as seleniferous wheat. Mortality was increased, and weight gains were decreased, as were feed intake and water intake. Hadjimarkos (1963) gave rats selenium at 3 ppm as sodium selenite and fluoride at 50 ppm as sodium fluoride. A second group received water with selenium at 3 ppm. The growth and mortality data indicated that the combined administration of selenium and fluoride did not increase the severity of signs of selenium toxicity.

Beneficial Effects. Selenium as an essential element. Diplock (1976) recently reviewed the multitude of studies concerned with the nutritional roles of selenium in laboratory and domestic animals and poultry. Selenium, the factor 3 of Baker's yeast, was effective in preventing hepatic necrosis in rats; exudative diathesis in chicks and turkey poults; skeletal muscle degeneration and necrosis in poultry, domestic animals (such as lambs, calves, and swine), and laboratory rodents; hepatitis dietetica in swine, and cardiac myopathy in turkeys, swine, sheep, and cattle. Other selenium deficiency diseases include reduced fertility, embryonic mortality, and unthriftiness in sheep and smooth muscle (gizzard) myopathy in turkey poults. Degeneration and necrosis of pancreatic acinar epithelium followed by fibrosis have been produced in chicks by selenium deficiency in the presence of adequate polyunsaturated fatty acids and vitamin E (Thompson and Scott, 1962).

Several reports have indicated that selenium has antitumor activity in some animal model systems, and these were reviewed by Shapiro (1972). Clayton and Baumann (1949) reported that the incidence of hepatic tumors induced by feeding of dimethylaminoazobenzene was decreased by about half by a diet containing selenium at 5 ppm. Shamberger (1970) found that selenium as sodium selenide greatly reduced the number of tumors in mice when administered concomitantly with croton oil to mouse skin treated with dimethylbenz (a) anthracene (DMBA). Shamberger (1970), in a study in which DMBA was applied to the shaved skin of ICR mice, found that sodium selenide (0.0005%) applied after DMBA reduced the incidence of papillomas. Sodium selenide applied concomitantly with 0.01% methylcholanthrene reduced the total numbers of papillomas and cancers, compared with controls. Dietary sodium selenite at 1 ppm markedly reduced the number of papillomas induced by the combination of DMBA-croton oil and by benzo[a]pyrene.

Mautner and Jaffe (1958) used experimental mouse tumor systems and reported that equimolar amounts of 6-selenopurine and 6-mercaptopurine produced similar degrees of inhibition of the growth of L1210 leukemia cells. 6-selenopurine was somewhat more toxic than 6-mercaptopurine at doses required to cause 50% (or greater) reduction in tumor size. Activity of 6-selenopurine against leukemia L5178-Y and sarcoma S-180 was less than that of 6-mercaptopurine, and the highest dose that caused a significant inhibition of tumor growth was markedly toxic. Mautner *et al.*

(1963) found that intraperitoneal and subcutaneous injections of selenoguanine inhibited the growth of L5178-Y lymphoma in mice. Riley (1968) studied the relationship between subcutaneous mast cell populations and papilloma induction and found that topical selenium decreased the incidence of papilloma and prevented the accumulation of mast cells at the base of the tumors in mice treated with carcinogen.

Analysis. In the last 20 years, much effort has been given to development of quantitative methods of analysis for nanogram amounts of selenium in a variety of materials. Methods include titrimetry, colorimetry, fluorometry, atomic absorption, polarograpy, and neutron activation. For samples containing large (microgram or milligram) amounts of selenium, titrimetry and colorimetry are usually satisfactory. Good microanalytic methods include fluorescence and atomic-absorption spectroscopy. Olson *et al.* (1973) and Watkinson (1967) have reviewed methods for determination of selenium.

Water. The current U.S. Public Health Service drinking water standard for selenium is 10 μg/liter (as total selenium); according to the 1975 Chemical Analysis of Interstate Carrier Water Supply Systems published by the EPA (1975), this concentration is rarely exceeded. Methods with sensitivities better than 10 ppb (10 μg/liter) are required for determinations in most drinking water.

The atomic-absorption method of Fernandes (1973) offers a highly sensitive and simple method of detecting nanogram quantities of selenium (and arsenic). The selenium in the water sample is reduced to gaseous hydride with sodium borohydride $NaBH_4$). The hydride is carried into an argon-hydrogen-entrained air flame with argon carrier gas. An atomic-absorption spectrophotometer with an electrodeless discharge lamp can have an absolute detection limit of 15 ppb based on a 20-ml sample volume. With a hollow-cathode lamp, the solution detection limit is 0.25 ppb (μg/liter). With either lamp, the method offers a precision (coefficient of variation) of 3%. The method detects both inorganic and organic selenium, and drinking-water samples offer few interfering compounds.

Polarographic procedures have been developed for selenium, but do not have the sensitivity and specificity of atomic-absorption methods.

Lambert *et al.* (1951) developed a colorimetric method for water analysis based on conversion of inorganic and organic selenium to selenious acid, which was then used to oxidize iodide quantitatively to elemental iodine; the iodine was determined colorimetrically. The method was subject to several interferences and could reliably detect selenium in water only at concentrations of 0.1 ppm (100 μg/liter) or more.

Greater sensitivity (by a factor of 10) is obtained with fluorometric methods of analysis. Selenium, as selenious acid, is complexed with 3,3-diaminobenzidine (Cousins, 1960) or 2,3-diaminonaphthalene (Parker and Harvey, 1961). Watkinson (1960) reported measuring less than 10 ng of selenium with a standard deviation of 0.5 ng, using 2,3-diaminonaphthalene fluorometric analysis. Iron and copper interfere with the analysis, and the technique is considerably more involved than the atomic-absorption method.

Biological Samples. The atomic-absorption method described above for drinking water can be applied to acid digests of biologic samples. Fluorescence methods are also widely used. The *Official Methods of Analysis of the Association of Official Analytical Chemists* (Horwitz, 1970) lists a 2,3-diaminonaphthalene fluorometric method for plant specimens containing selenium at less than 4 ppm; the analysis takes several hours to complete. For plants containing larger concentrations of selenium, the AOAC uses a colorimetric method, precipitating the distilled selenium with hydroxylamine hydrochloride and measuring the selenium concentration against prepared standards with a color comparator. For seleniferous plants in which the selenium concentration exceeds 100 ppm, a gravimetric method is listed by the AOAC.

For food samples, the official AOAC final action method is an [illegible] digestion in the presence of a mercuric oxide fixate. The selenium is distilled as the bromide and reduced to elemental selenium with sulfur dioxide, and its concentration is determined as selenious acid, H_2SeO_3, by titration with sodium thiosulfate, and iodine.

Neutron-activation analysis has been used successfully to measure selenium in biologic samples. Irradiation of the sample with neutrons in a nuclear reactor produces many radioactive elements, including selenium. Three radionuclides of selenium are produced at sufficiently high specific activity to be useful for determinations in biologic samples. The half-lives of these radionuclides are 120 days for selenium-75, 17.5 for selenium-77, and 18.6 min for selenium-81; the first two are gamma emitters and selenium-81 is a beta-emitter (Bowen and Cawse, 1963). Isolation of a specific radionuclide can require much time and effort and is generally limited to waiting several weeks for the short-lived elements to decay away, spectrometrically analyzing for gamma-emission peaks at 121 + 136 and 265 + 280 keV (Grant *et al.*, 1961), distilling the selenium with hydrogen bromide or extracting into an organic solvent the selenium complex of diaminobenzidene or diaminonaphthalene (Betteridge, 1965). Such techniques are reported to detect as little as 10 ng of selenium per sample.

Reliability. Before 1960, most water samples were analysed by colorimetric or titrimetric methods for selenium, and these methods are of low sensitivity by today's standards. In fact, the minimal detectable concentrations by these methods are higher than the drinking-water standard of 10 µg/liter. Water analyses completed before 1960 are likely to contain false-negative results that is, reporting no detectable selenium when, in fact, the drinking-water standard of 1962 may have been exceeded.

Methods requiring prolonged air drying of the sample, especially at temperatures above 60° C, or requiring distillation are subject to loss of selenium which results in underestimation of the selenium content. Many compounds have the potential of interfering with selenium determinations; iron, copper, and arsenic seem to offer the most problems in water analysis, but are well accounted for in the colorimetric method of Lambert *et al.* (1951) and its modifications.

Conclusions and Recommendations. The determination of a "no-adverse-effect" concentration of selenium is complicated by numerous experimental variables. The toxic effect of selenium depends on the type of selenium compound administered, whether it is organic or inorganic, the valence state of the selenium ion, the species and sex of the laboratory animal used, the age of the animal, the conditions and duration of the test, and the diet--whether natural or semipurified ingredients, the protein content, the caloric intake, the type of protein, and the presence and concentrations of other elements, such as arsenic, mercury, thallium, and fluorine. The criterion of toxicity used is also important in establishing a no-effect concentration and growth may be the best indicator of toxicity.

Harr and Muth (1972) used a semipurified diet in rats and reported that the minimal toxic concentration for induction of hepatic lesions was 0.25 ppm. A concentration of 0.75 ppm was considered the minimal concentration, with respect to longevity and development of cardiac, renal, and splenic lesions. However, rats fed selenium at 0.5 ppm grew as well as control rats. Halverson *et al.* (1966), in a study of rats and wheat diets, found that selenium at 3.2 ppm as sodium selenite did not affect growth over the feeding period of 6 weeks. Thapar *et al.* (1969) reported that a dietary selenite concentration of 2 ppm had no detrimental effect on chickens fed over a life cycle.

Although there is now general agreement that selenium is an essential element for man as it is for domestic animals, virtually nothing is known about the forms and quantities of selenium consumed by man, in part because of inadequate methodology for collecting material and for accurate analysis. This must be corrected before the environment (water, air, food) can be satisfactorily monitored.

Metabolism and kinetics of the various forms of selenium require intense research efforts. Molecular transformations must be determined in mammalian systems and the interactions between selenium and other environmental materials, particularly mercury, cadmium and arsenic.

Little information is available as to effects of long term exposure to relatively low levels of selenium. Although selenium is toxic to man and animals in high doses, these are usually a result of accidental exposure. Rather than concern for toxicity the literature indicates that there is a greater potential for a deficiency. Consideration should be given to raising current permitted levels in waters of the United States.

The paucity of definitive data on selenium and human health requires a number of approaches toward elucidating the role of selenium in the mammalian system. The following recommendations are suggested:

1. There is a critical need for more rapid, accurate, and reproducible analytic methods that will permit both qualitative and quantitative assays. Information on chemical forms, oxidation states, and solubility in water is needed. This is probably the most limiting need for progress over a broad front in selenium research.
2. Systems for monitoring the environment (water, air, food) should be improved.
3. Basic research should be conducted to define molecular transformations in the mammalian system.
4. Effects of selenium on the toxicity of mercury, cadmium and arsenic should be studied.
5. Natural and industrial emission and cycling of selenium in the environment should be investigated.
6. The effects on animal systems of long-term low concentrations of selenium in and in combination with other trace elements in the environment should be determined.
7. Baseline data on selenium concentrations in humans in health and disease are needed.
8. The effects of selenium deficiency and excess on induced and spontaneous animal tumors should be determined.

Fluoride

Occurrence. Fluorine is the most electronegative of all elements, existing naturally in the form of fluoride. It is the 17th most abundant element in the earth's crust, occurring principally as fluorite, (CaF_2) and fluoroapatite, ($Ca_{10}(PO_4)_6 F_2$). It is present in small amounts in most soils except those that have been strongly leached. The concentration of fluoride in natural waters depends principally on the solubility of the fluoride-containing rocks with which the water is in contact.

In 1969 the general Community Water Supply Survey of the Public Health Service sampled 969 water supplies and found flouride ranging from less than 0.2 up to 4.40 mg/liter. Fifty-two systems had fluoride concentrations greater than the then-recommended limits for this constituent.

A more extensive survey in the same year by the Dental Health Division of the Public Health Service (1970) showed that 8.1 million people in 2,630 communities in 44 states were consuming water with more than 0.7 mg/liter of naturally occurring fluoride. Nearly one million people in 524 communities were receiving water with more than 2 mg/liter of naturally occurring fluoride.

Most of the communities with more than 0.7 mg/liter natural fluoride were in: Arizona, Colorado, Illinois, Iowa, New Mexico, Ohio, Oklahoma, South Dakota and Texas. There were no reports of community water supplies with as much as 0.7 mg/liter fluoride from Delaware, Hawaii, Massachusetts, Pennsylvania, Tennessee or Vermont.

The WHO monograph "Fluorides and Human Health" (1970, pp 17-59) notes that high concentrations of fluoride are found in areas of every continent and that dental fluorosis is a problem from Finland to South Africa and from England to Japan.

Fluoride in Water Treatment. Conventional procedures of water treatment, i.e. clarification, filtration, softening, disinfection, have little or no effect on the fluoride concentration in water. However, it was noted in Ohio in the 1930's that if the pH was increased during softening operations to a value high enough to precipitate magnesium hydroxide, some removal of fluoride was accomplished, (Scott et al., 1937). In most full-scale plants the removal of fluoride was less than 50 percent.

Two special processes have been used for fluoride removal, both based on the adsorption of fluoride on granular media, either activated alumina or bone char (Maier, 1953). The water containing fluoride is passed through a bed of the medium until the effluent concentration exceeds an acceptable value. The medium is then regenerated with a solution of sodium hydroxide to remove adsorbed fluoride. Costs of this type of treatment are such that few communities have undertaken voluntarily to remove fluoride from their supplies.

Addition of Fluoride to Water Supplies. For more than 30 years, the practice of adding fluoride to drinking water has been practiced in the U.S. for reduction of dental caries. The principal chemicals used for this purpose are sodium fluoride, sodium silicofluoride, hyrofluorosilicic acid and ammonium silicofluoride (Maier, 1963). These chemicals require care in handling and must be dispensed with properly designed chemical feeding systems, but these are readily available. The usual dosage has been in the range of 1 mg/liter.

When any one of these chemicals is dispersed in water at the 1 mg/liter range, it dissociates essentially completely almost completely to yield fluoride ion.

Other Sources of Fluoride

Food. Fluoride is present to some extent in nearly all foods, but the concentrations vary widely. Studies of the fluoride contents of foodstuffs reported in the WHO Monograph (1970) have been reviewed by Muhler (1970). Prival and Fisher (1973) have made a more recent compilation of these fluoride contents.

Among the foodstuffs notably high in fluoride are fish, particularly those, such as sardines, that are eaten with the bones. Fish meal flour, which is produced from the whole fish, is also high in fluoride. Tea is unusually rich in fluoride. Milk and most fruits are generally low in fluoride. Vegetables vary greatly in fluoride content.

Hodge and Smith (1965) computed the total fluoride intake from food at 0.5 - 1.5 mg/day for areas with nonfluoridated water. Marier and Rose (1966) showed that use of fluoridated water in canneries increased the fluoride content of canned food by 0.5 mg/liter and converted this to 0.5 mg/day in the diet. The proposed total intake from the diet then became 1.0 - 2.0 mg/day.

However, the Hodge and Smith estimate came from Machle and Largent (1943) whose values were based erroneously on earlier work (Machle *et al.*, 1942). In this earlier work the average total fluoride intake per day for 70 weeks was just under 0.5 mg with only 0.16 mg of the intake from food as such. In a more recent reveiw Hodge and Smith (1970) have lowered their estimate to 0.3 - 0.8 mg fluoride daily from the diet.

Recent studies indicate that the total intake of fluoride is as high as 3 mg/day rather than the earlier figure of 1.5 mg/day, primarily because of increases in the estimated levels of fluoride in foods (Spencer *et al.*, 1970). Balance data presented by Spencer also suggest a higher retention by bone, nearly 2 mg/day rather than the 0.2 mg/day indicated earlier.

Two recent articles from Spencers group (Kramer *et al.*, 1974; Osis *et al.*, 1974) appear to support a higher estimate for dietary fluoride intake. The first is based on hospital-prepared food from 16 U.S. cities. The fluoride intake from food in the fluoridated communities was found to range from 1.6 - 3.4 mg/day (av. 2.6) while that from nonfluoridated cities was 0.8 - 1.0 mg (av. 0.9). The very high values and the marked difference between fluoridated and nonfluoridated cities can be explained in part by the inclusion of coffee and other water-based beverages as dietary intake, a classification not usually followed by other investigators. The second article reports average fluoride intake from diets used in balance studies in a fluoridated city over a six-year period as 2.0 mg/day.

These findings are important because if valid, they might represent a shift in intake that could lead to dental fluorosis in fluoridated communities. Also, a retention of 2 mg/day would mean that an average individual would experience skeletal fluorosis after 40 years, based on an accumulation of 10,000 ppm fluoride in bone ash. However, these new estimates for fluoride in food are questionable, consequently, so are their implications. The values are suspect because of analytical problems. The diffusion method of Singer and Armstrong (1969a) was used with a colorimetric reagent and false high values are obtained with this technique (Taves, 1966).

A study more limited in scope, because it was restricted to 16 to 19 year old males, found 2.0-2.3 mg/day total fluoride intake (San Filippo and Battistone, 1971). The increase over earlier values may reflect the fact that the food portions were large for the test group.

Data from balance studies in children tend to support the lower values. The dietary fluoride intake for nine children aged 4 to 18 years averaged 0.3 mg/day (Forbes *et al.*, 1973).

The quickest and most reliable method of checking whether there has been a shift in total intake of fluoride in the past 20-30 years is through surveys of the urinary and bone fluoride concentrations occurring in people in fluoridated communities.

There has been no question about the analytical techniques used in these earlier data on urine and bone because the concentrations involved were relatively high. A recent (Parkins, 1974) bone survey in Iowa done on autopsies showed bone fluoride levels higher than those in earlier publications, particularly when taking into account that they are for washed bone, which means that the concentrations need to be approximately doubled to compare them to values for ashed bone. Detailed comparison of the method he used has shown no systematic error, but other bone fluoride values found in Rochester, New York, show concentrations which match earlier values almost exactly (Charen et al., in preparation, 1976).

Private communication with Parkins has not clarified the discrepancy, but he has indicated that the usual fluoride concentration in the urine is about 50 µM, which is the same as earlier reports as well as the values found in a few samples analysed from Rochester residents. Obviously additional work is desirable to clarify these questions, but earlier values for average fluoride intake and balance still appear to be valid.

Industrial exposure. Industrial exposure to fluoride-containing dusts and gases has been a serious problem in many parts of the world. A committee of the National Academy of Sciences on the Biological Effects of Atmospheric Pollutants (1971) reported on fluoride as an atmospheric pollutant both in work place and in the ambient air.

Operations that introduce fluoride dusts and gases into the atmosphere include: grinding, drying and calcining of fluoride-containing minerals; acidulation of the minerals; smelting; electrochemical reduction of metals with fluoride fluxes or melts as in the aluminum and steel industry; kiln firing of brick and other clay products and the combustion of coal.

Generally speaking, good progress has been made in reducing fluoride exposure to industrial workers by ventilation and emission control practices.

Air. Air pollution by fluoride dusts and gases has done substantial damage to vegetation and to animals in the vicinity of industrial fluoride sources. However, the contribution of ambient air to human fluoride intake is only a few hundredths of a milligram per day (NAS, 1971), an amount that is insignificant in comparison with other sources of fluoride.

Metabolism. Radiofluoride studies show metabolism of fluoride in the body to be simple. Hence, fluoride is unlikely to give rise to intolerance by reason of disease or genetics. Accumulation has been found to occur only in the kidneys and calcified tissues. The reabsorption of water without the reabsorption of fluoride in the kidneys explains the increased concentration in the kidneys. The isomorphism of the fluoride ion for the hydroxyl ion in hydroxyapatite explains the increased concentration of fluoride in calcified tissues. Some of the fluoride ingested is retained in the calcified tissues; however, the rate of such retention decreases with age, so adults are nearly in balance (Hodge, 1961).

There has long been confusion about the relationship of serum fluoride concentration to intake, confusion that carries over to recent reviews. Some 24 years ago, Smith, Gardner, and Hodge found a 2-3 fold increase in the average serum fluoride concentration of people living in a fluoridated community, compared to those in a nonfluoridated community (0.7 vs. 1.8 µM, or 0.014 vs. 0.036 ppm) (Smith *et al.*, 1950; see Taves, 1966 for revised values). Such a relationship would be expected, if about 0.5 mg of fluoride per day were coming from food in the nonfluoridated community and 1.5 mg from food and water in the fluoridated area.

This work was ignored by Singer and Armstrong (1960) when they developed what appeared to be improved analytic techniques. They found more fluoride in the serum (7.5 µM, or 0.15 ppm), but found no differences related to intake, although concentrations in the drinking water varied from 0 - 2.5 ppm. No adequate explanation was offered as to how the urinary and bone concentrations could be directly related to the intake of fluoride, while serum concentrations remained constant. Threshold phenomena in both kidney and bone would have to be present to maintain a constant serum concentration, but there was no reason to think that a threshold existed in either case. The exchange and uptake into the bone compartments cause a buffering of the serum fluoride concentration, both short-term (days) and long-term (years). However, this does not explain why the average serum fluoride concentration would be the same in different communities. Even though buffering would tend to diminish the surges of serum fluoride due to ingestion, the mean value should be an integrated reflection of the average intake and bone fluoride stores.

The explanation for the confusion is to be found in the presence of two forms of fluoride in human serum, one of which can be shown to exchange with added radioactive fluoride and one which cannot (Taves, 1968a,b). The exchangeable fraction is smaller and is the same as ionic fluoride. Ionic fluoride can be measured directly with a fluoride electrode reasonably well, and even more accurately after diffusion or ultrafiltration. It can also be measured with a fluorometric reagent. The renal clearance of this fraction coincides with the renal clearance of radioactive fluoride (Taves, 1967), and also varies with the fluoride concentration in the water supply (Guy *et al.*, 1976).

With fluoride content of water at 1 ppm, the serum inorganic fluoride content is on the average less than 1 µM, or 0.02 ppm (Taves, 1966; Singer and Armstrong, 1973; Hanhijarvi *et al.*, 1972). This contrasts with the average value of 0.15 ppm found by Singer and Armstrong after ashing and distillation, a value that should now be labeled "total" fluoride. In other words, variations in serum inorganic fluoride were hidden by a larger fraction of organic fluoride, which was being measured along with inorganic fluoride when ashing was employed.

Guy *et al.* (1976) reported the isolation of an 8-carbon perfluorinated compound from human plasma. If the second form of fluoride proves to represent only chemicals of this type with no relation to fluoridation, the case for the safety of fluoridation will have been strengthened, in that the behavior of inorganic fluoride in the body will have become simple and understandable. Whether food is the source of this second form of fluoride is unclear, in part because the reports of organic fluoride in food have not been confirmed (Weinstein *et al.*, 1972). There is heavy use of fluorocompounds industrially and in homes (Bryce, 1964). This may explain its presence in humans.

Under steady-state conditions, at least 99% of the fluoride present in the body is sequestered in calcified tissues. Most of the remainder is present in plasma and is thus available for excretion. Hodge (1961) has emphasized that skeletal sequestration and renal excretion are the two major means by which the body prevents the accumulation of toxic amounts of fluoride ion.

Chen *et al.* (1956) measured the renal clearance of fluoride in female dogs. After showing that fluoride was completely ultrafiltrable in dog plasma, they used routine clearance procedures in animals drinking tap water containing fluoride at 1.0 ppm (artificially fluoridated). The average normal renal fluoride clearance was 2.7 ml/min, and the fluoride: chloride clearance ratio was 19:1. During mannitol diuresis, fluoride clearance varied directly with the urinary flow rate. Hypertonic sodium chloride infusion also increased the clearance of fluoride. Although the authors claimed that the intravenous administration of sodium nitrate or sodium sulfate did not affect fluoride excretion, their data did not establish that point. Plasma fluoride concentrations in their experiments ranged from 12 - 61 µg/100 ml.

Carlson *et al.* (1960a) studied the renal excretion and clearance of radiofluoride in dogs. Reabsorption ranged from 14 to 92% of the filtered load and was consistently less than reabsorption of chloride. Renal clearance of fluoride varied directly with urinary flow rate and with plasma concentration. There was no indication that renal tubular secretion occurred.

In a later study, Carlson *et al.* (1960b) fed 1 mg of radiofluoride to each of two adult humans. Fluoride clearance always exceeded chloride clearance and increased with urinary flow rate. Fluoride clearance was always smaller than creatinine clearance. Although less than 10% of the ingested radioisotope was present in the plasma volume at any time, about one-third of the ingested dose appeared in the urine within 4 hours. Chlorothiazide, a benzothiadiazine diuretic, increased the clearance and excretion of fluoride. Plasma contained 72% of the whole-blood fluoride. This differential distribution between plasma and red cells was also observed in dogs. (Carlson *et al.*, 1960c).

More recently Whitford *et al.* (1976) have demonstrated that the renal clearance of fluoride is inversely related to tubular fluid pH. They showed that urine flow rate and chloride clearance which were previously thought to be the main determinants of fluoride clearance, were not strongly associated with fluoride clearance.

Walser and Rahill (1965) have shown that the renal tubular reabsorption of iodide, bromide, and fluoride is related to the simultaneous reabsorption of chloride. The results indicate that all the halides are reabsorbed predominately by the same mechanisms. If reabsorption of fluoride is achieved by utilization of renal chloride transporting systems, chloride must be the preferred substrate because the clearance of fluoride is generally much greater than the simultaneous clearance of chloride.

A summary of the findings from these studies on renal handling of fluoride is:

1. Virtually all the fluoride in plasma (human or dog) is ultrafiltrable.
2. Renal excretion of radiofluoride depends on glomerular filtration and variable tubular reabsorption.
3. Probably, reabsorption is largely passive, with fluoride being less permeable than chloride.
4. Fluoride excretion increases when the plasma concentration is increased.
5. Procedures that increase urinary flow rate (e.g., administration of osmotic diuretics, hypertonic saline, or diuretic drugs) increase the clearance of fluoride.

HEALTH ASPECTS

Acute effects. Acute toxicity from fluoride is quite rare and occurrs principly as a result of suicide or accidental poisoning. The lethal dose of sodium fluoride for man is about 5 g, but there are reports of recovery from amounts much greater as well as deaths from smaller quantities (Goodman and Gilman, 1975).

Initial symptoms of toxicity are a result of the local action of fluoride on the mucosa of the gastrointestinal tract. Vomiting, abdominal pain, nausea and diarrhea are followed by paresthesias, hyperactive refexes and tonic and clonic convulsions. No system of the body can be considered exempt and death is usually due to respiratory paralysis or cardiac failure. Many of the signs and symptoms of acute fluoride toxicity are a result of the calcium-binding effects of fluoride (Goodman and Gilman, 1975; WHO, 1970).

Chronic Effects. Chronic toxicological studies indicate that teeth and bone are the most fluoride-sensitive tissues. The margin of safety with fluoridated water (assuming an intake of 1 mg/day) has beeen estimated to be 2-8-fold for dental mottling. Years of experience with fluoridation without apparent objectionable mottling (see below for possible exceptions) attest to the adequacy of this low margin of safety. Crippling skeletal fluorosis has a 20 to 40-fold margin of safety for the average person, again assuming an intake of only 1 mg/day (Hodge, 1961 and 1962).

Epidemiological studies where the water is naturally high in fluoride have reported no adverse effects, except in rare cases, until the concentration is many times that recommended for artificial fluoridation (Hagen *et al.*, 1954; Leone *et al.*, 1954; AMA, 1957). Indeed, there is a suggestion that 1-5 ppm may prevent bone loss to some degree and decrease the amount of soft tissue calcification in older people (Bernstein *et al.*, 1966).

Controlled studies with recommended levels of added fluoride, such as in Kingston-Newburg, have reported no evidence of adverse effects (Ast *et al.*, 1956).

Mongolism. The possibility that mongolism is caused by fluoride in the drinking water stems from a report by Rappaport (1959) in which he observed a dose-related association between the number of cases of mongolism registered in institutions and the concentrations of fluoride in the water. From the towns with less than 0.1 ppm to those with 1.0-2.6 ppm, the increase was nearly 3-fold. This study has been criticized because the case rates were less than half those found in intensive case-finding studies (Royal College of Physicians, 1976).

Three intensive case-finding studies in Britain (Berry, 1958, and two unpublished ones cited by the Royal College of Physicians, 1976) with different fluoride concentrations in the water have not shown such an association. Heavy tea-drinkng in England (Cook, 1970) might obscure differences in the British studies. However, the absolute rates were similar to those in a recent intensive case-finding study in Massachusetts (Needleman et al., 1974), in which no difference was noted between fluoridated and nonfluoridated communities. Therefore, for Rappaport's hypothesis to be maintained, an explanation as to why the British rate did not reflect increased consumption of fluoride from tea dinking would be necessary. Needleman estimated that he could have detected an increase of as little as 20% at the 95% confidence level. He admitted that his evidence was not adequate to rule out an effect where fluoridation had been present for the lifetime of the mother, because his data involved a relatively short period of fluoridation.

Sensitivity To Fluoride. A recent report (Grimbergen, 1974) suggests confirmation of the earlier claims by Waldbott (1962) that some people are very sensitive to fluoride. Waldbott's claims have been dismissed on two grounds: that he was the only one to report such effects, and that sensitivity of this type has not been reported among the billions of tea drinkers in the world who would be ingesting extra fluoride (WHO, 1970, p. 15).

Grimbergen's report was a preliminay methodological paper and is not convincing. Two aspects of the methodology seem weak and could lead to erroneous conclusions. First, when large numbers of double-blind tests are done, it is to be expected that control patients will occasionally have symptoms that correspond to those associated with the administration of fluoride; the investigator should indicate the rate of positive responses and the results of retesting. Second, the patients themselves were adding highly concentrated solutions to their drinking water, so it is possible that they were curious and broke the codes by taste or other means. Waldbott's case reports (1962) are more completely documented and he used concentrations that were probably too low to be identified by taste. He reported 29 positive responders among 48 people tested. The Royal College of Physicians (1976, p. 63) review stated that sodium fluoride at 1 mg/15 ml of distilled water has a distinctive taste. However, Taves (unpublished, 1976) found that four people out of five could not tell the difference at 1 mg/15 ml.

Waldbott and Grimberger are not the only ones who have described patients with syndromes that they explained as intolerance to fluoride. Douglas (1957) tested 32 patients in a group of 133 with histories suggestive of sensitivity to fluoride-containing dentifrices. He implied that none were able to complete a series of six alternating trials using fluoride and nonfluoride toothpastes, because of intolerance, mainly in the form of ulcerations of the mouth. Feltman and Kosel claimed that, among pregnant mothers and their children, 1% (at least four of them) reacted adversely to 1 mg fluoride tablets. They stated that they established (by means of placebos) that it was the fluoride, rather than the binder, that caused the adverse effect (Feltman, 1956; Feltman and Kosel, 1961). Shea, Gillespie, and Waldbott (1967) reported on seven cases of patient improvement after discontinuing vitamin drops or toothpaste containing fluoride. They subjected one case to a double-blind study with sodium fluoride. In the cases involving toothpaste, the associated cation is not stated. Stannous fluoride is commonly used in toothpaste; therefoe, sensitivity to tin, rather than to fluoride, cannot be ruled out. Petraborg (1974) reported on seven case histories of what seemed to be fluoride sensitivity, but the patients were not subjected to objective tests, so the evidence is weak.

The quantities of fluoride involved are clearly relevant to the question of the safety of fluoridation. But, if Feltman and Kosel's estimate of 1% intolerant people is correct, there should have been more reports of adverse effects in the studies in which fluoride tablets were given to schoolchildren (at least 10,000 children by 1967, mainly in Switzerland) (O'Meara, 1968). Also, as methoxyflurane anesthesia for surgery typically causes serum fluoride content to increase to 30-50 times normal (Fry *et al.*, 1973), there should have been striking cases of such intolerance in an estimated 12 million patients who have recieved methoxyflurane (NAS-NRC, 1971). Moreover, cases of intolerance to fluoride (20-100 mg/day) for osteoporosis have been associated with very few symptoms of the type reported by Waldbott. There have not been reports of intolerance from people who move into and out of numerous towns with naturally high fluoridated water supplies. Opportunities for such discovery existed before any bias for or against fluoridation.

So, although sensitivity to fluoride has not been demonstrated firmly, a possibility of sensitivity or idiosyncratic reaction to fluoride should be kept in mind. Clarification might come from two kinds of study. Studies on the administration of fluoride drops or tablets for prevention of dental caries should include consideration of possible intolerance and definite statements should be made about any findings in this regard; in most such reports, no comments are made about a search for intolerance. Quissell and Suttie (1972) have demonstrated an ability of fluoride-resistant cells to remove or exclude fluoride from their interiors in vitro. Humans or animals receiving fluoride for long periods should be studied to see whether a cellular resistance develops _in vivo_. If this could be demonstrated, the metabolic consequences of resistance or its absence might shed light on how intolerance could occur.

Renal Patients. The effect of impaired renal function on the handling of fluoride could not be adequately studied until accurate measurements of serum ionic fluoride could be made. Evidence that was thought to carry some weight in the past, such as the lack of observed increase in serum concentration and the similarity of urinary fluoride concentrations in patients with renal disease, is faulty. Difficulties with serum fluoride values have already been discussed. Urinary concentrations will reflect impaired renal ability to excrete fluoride only if there has not been sufficient time for equilibrium with fluoride intake to be reached.

Three reports confirm the belief that renal patients have a lower margin of safety than the average person. Hanhijari _et al._ (1972) noted that serum fluoride concentrations in patients with renal disease were as much as five times greater than in normal people. The increase can be explained on the basis of decreased renal clearnace of inorganic fluoride (Berman and Taves, 1973). One case of symptomatic skeletal fluorosis (radiculomyelopathy) has been reported from an area in Texas with natural fluoride at 2.3-3.5 ppm in the water. (Sauerbrunn _et al._, 1965). There have been two cases of suspected skeletal fluorosis (based on X-ray evidence) in the United States with fluoride at 2-3 ppm in the drinking water (Juncos and Donadio, 1972). The combination of renal impairment and very high water intake was thought to account for these findings.

The best available information on the implications of increased serum fluoride concentration in renal patients is from studies on patients requiring renal dialysis. In these patients, serum fluoride during dialysis rises to about 25-30 μM; consequently, any effects should appear earlier than in renal patients not on dialysis. A double-blind study of the effect of fluoridated dialysate in long-term hemodialysis has been conducted by deionizing the water and adding fluoride or chloride via coded ampules to the dialysing water of the artificial-kidney machine. Twenty patients were investigated for over a year (average, 20 months). Patients on fluoride showed no differences, when compared with the control group, except for the histologic observation of increased thickness of trabecular bone (Oreopoulos _et al._, 1974).

The question of effects on patients who are maintained on dialysis for longer perods, or who are not yet adults, is certainly not settled. The fasting (5-10 μM) and maximal (25-30 µM) serum fluoride concentrations in hemodialysis patients are almost identical with concentrations in patients being treated wih fluoride for various bone diseases (Taves, unpublished data); hence, some effect is possible.

The implications, however, of bone effect (fluorosis) from excess fluoride are not clear, unless a limitation of joint movement or of exit of the spinal nerves occurs. Fluoride is being used deliberately to produce increased bone density in patients with bone disease; therefore, it may be that small increases in bone density in renal patients may be advantageous, rather than harmful. Whether treatment with fluoride will result in improved bone density may be determined by the interrelated metabolism of calcium and vitamin D (Jowsey *et al.*, 1968). Because calcium metabolism is altered in patients with renal disease, a beneficial effect of long-term fluoridated-water hemodialysis may not be automatic, even if fluoride proves to be beneficial in other bone diseases or in preventing fractures due to osteoporosis.

The possibility of adverse effects in renal patients still dependent on their own kidneys cannot be entirely ruled out by a demonstration of benefit to those on dialysis. The possibility of adverse effects of fluoride on marginally functional kidneys has to be considered for patients with renal failure, but two lines of evidence make adverse effects unlikely. Considerable information has been collected on serum concentrations that will cause functional renal changes. This was the rerult of evidence that inorganic fluoride from the metabolism of methoxyflurane was the cause of the polyuria occasionally seen after the use of that anesthetic. The serum concentrations of inorganic fluoride necessary to cause polyuria were found to start at about 40-50 µM, and severe effects required 150 µM. The immediate cause of the polyuria is the loss of the electrolyte concentration gradient from the cortex to the papilla (Whitford and Taves, 1973).

Cancer. Early in 1975 it was claimed by Yamouyiannis that there is a linkage between fluoridation of water and increased cancer rates. The initial data presented and shown in Table V-15 are the sum of rates for nine specific cancer sites (seven for white males and two for white females) for the ten largest cities with fluoridated water supplies for more than 12 years prior to 1970 and for the ten largest nonfluoridated U. S. cities. The source of the data was the age-specific cancer rates for a 20-year period by site and county compiled by the National Cancer Institute (NCI) and published by the Department of Health, Education and Welfare (HEW) in 1974. There is clearly a difference between the two groups of cities, with the fluoridated ones having about 25/100,000 more cancer deaths than the nonfluoridated ones.

Later, in September 1975, Yamouyiannis and Burk submitted data to NCI suggesting that there had been a change in cancer rates with time for fluoridated cities as compared with nonfluoridated ones. A later version of the same data is shown in Figure V-1, where average annual crude cancer mortality rates have been plotted as a function of time for the 10 largest fluoridated cities and for 10 control cities, selected from among the largest nonfluoridated cities giving average crude death rates prior to 1952, for both groups. As can be seen, the average crude mortality rates diverge markedly after 1952, when the one group of cities initiated fluoridation.

TABLE V-16

Selected Cancer Mortality Rates for the Largest Cities by Water Status

Fluoridated		Non-Fluoridated	
Chicago	121.0	Los Angeles	98.3
Philadelphia	124.6	Houston	82.7
Baltimore	119.2	Boston	123.1
Washington	113.1	New Orleans	104.1
Cleveland	121.9	San Antonio	84.2
San Francisco	119.9	San Diego	85.6
Milwaukee	125.9	Seattle	96.7
St. Louis	119.3	Cincinnati	115.2
Pittsburgh	112.1	Memphis	83.4
Buffalo	121.6	Atlanta	85.8
Mean	119.9		95.5

(from Yiamouyiannis, 1975)

In considering this evidence the National Cancer Institute found that Yamouyiannis and Burk had failed to take into account differing demographic factors and age distributions that affect cancer rates. When the NCI used 1950 rates for the U.S. population as a whole to adjust the crude mortality rates of Figure V-1 for sex, race and age and expressed the results as the ratio of observed deaths to expected deaths (standard mortality ratios, S.M.R.) the time trends are eliminated as shown in Figure V-2.
The S.M.R.'s are greater than unity for both sets of cities and greater in the fluoridated than in the nonfluoridated ones, but there is no change in these ratios with time from 1950 to 1970 (Hoover *et al*., 1976).

In supplementary studies, NCI (1975) investigated the absolute differences in cancer rates in fluoridated and nonfluoridated areas. One study, results of which are shown in Table V-17, dealt with reasons for the differences shown in Table V-16. Regression Analyses was conducted for each of the nine cancer sites and the counties of the cities listed in Table V-16 using fluoridation as an independent variable alone and then after correction for a number of demographic variables. As shown in Table V-17 columns 3 and 4, the slopes of the regression lines (B), are generally positive and the F values are generally greater than 6 when only fluoridation status is considered, in agreement with the claim of Yiamouyiannis. When demographic variables are taken into account, however, the F values, given in the last column, become insignificant except for stomach cancer. Further regression analysis, allowing also for control of the specific high-risk ethnic groups for cancer, yielded a nonsignificant F value of 0.02 for females with only the F value for males, 6.9, remaining greater than the 95% confidence level. Since one positive correlation of this sort occurs by chance in 5% of cases examined and 16 cases were examined in this instance, this result cannot be regarded as strong evidence of a linkage between fluoridation and cancer.

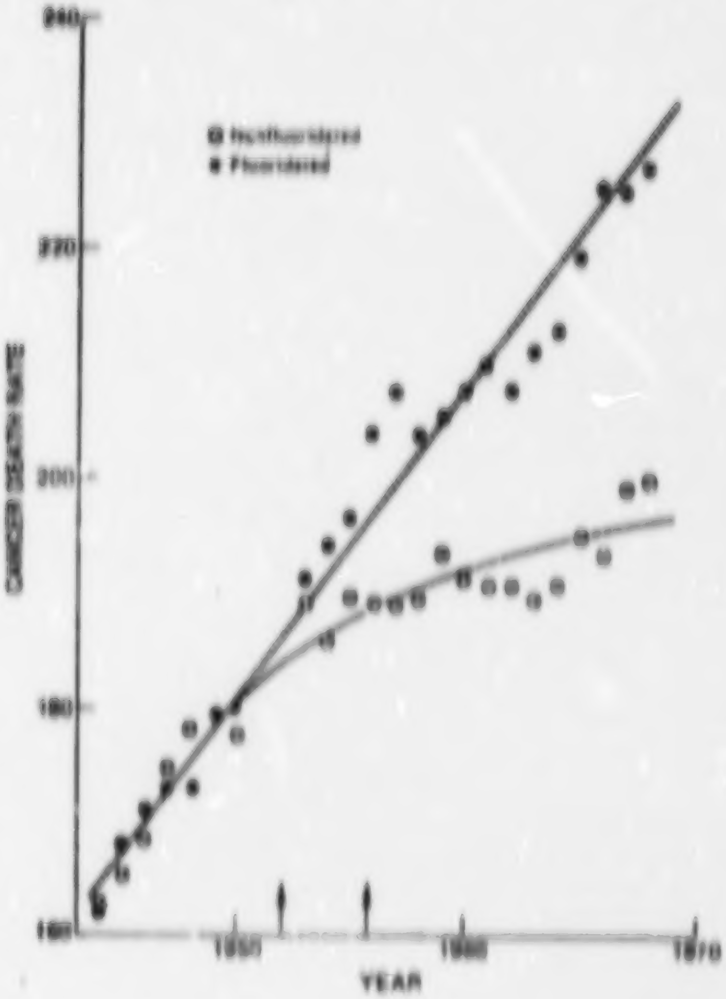

Figure V-1. Average crude cancer death rates for the 10 fluoridated cities from Table V-16 and 10 non-fluoridated cities selected from the 15 largest ones to match the pre-fluoridation period, 1944-1950. The two arrows mark the time when fluoridation was instituted in those cities. Reprinted, with permission, from Yiamouyiannis and Burk, 1975.

TABLE V-17

Regression coefficients (β) and F values associated with a fluoride variable entered into a regression analysis to predict sex and site-specific cancer mortality rates in 20 counties with and without control for demographic risk factors.

	Sex	Without Control β	Without Control F	With Control β	With Control F
Mouth and Throat	M	1.18	2.6	-0.78	0.8
	F	-0.16	1.9	-0.13	0.9
Esophagus	M	2.55	25.3	0.57	1.2
	F	-0.08	0.5	-0.29	2.6
Stomach	M	5.02	19.7	2.31	11.6
	F	2.22	11.1	0.79	7.6
Colon	M	5.67	23.1	0.64	0.3
	F	3.57	13.3	-0.31	0.1
Rectum	M	3.72	21.5	1.12	1.1
	F	1.50	17.5	0.23	0.2
Breast	F	3.85	16.1	0.76	0.2
Ovary	F	1.18	9.8	0.69	2.3
Kidney	M	0.66	7.2	0.11	0.2
	F	0.21	8.2	0.07	0.7
Bladder	M	1.49	13.1	0.64	1.3
	F	0.36	8.9	0.26	2.2

(from Hoover, 1975)

Some linkage may not be unreasonable, however, for fluoride will exist primarily as hydrofluoric acid, a highly penetrating and irritating chemical, in the acidic stomach. Hydrofluoric acid is also possibly mutagenic in plants (Mohamed, 1969) and drosophila (Mohamed and Kemmer, 1970). This will be considered more fully in a later section.

Two other studies by NCI (1975) give additional information on a possible linkage of fluorides to stomach cancer. In the first of these, cancer data for all the U.S. counties in which at least 2/3 of the population was first fluoridated between 1950 and 1965 were grouped into 5-year intervals in order to study changes with time. Results are shown in Table V-18 in terms of standard mortality ratios at some particular sites. The bone and kidney are of special interest because fluoride is concentrated in those tissues.

None of the specific sites give any indication of an increase in cancer following fluoridation; rather a possible decrease is suggested. The "other" category is of interest as the only grouping which suggested a possible increase for both sexes.

The other study compared naturally fluoridated and nonfluoridated counties in Texas on the same basis as in the previous one. Results are shown in Table V-19. The S.M.R.'s are more variable because of the smaller numbers involved, but there are no consistent trends wwith increasing fluoride content except for a possible decrease in the "other" category.

Thus, there is no confirmation of the hypothesis that fluorides or fluoridation causes cancer.

TABLE V-18

SMR's and number of deaths* in fluoridated counties in five-year intervals (pentads) related to pentad of fluoridation

Site:		Stomach		Kidney	
Sex:		Men	Women	Men	Women
Prior to Fluoridation:					
	10 yrs.	1.0 (352)	0.9 (205)	1.1 (76)	1.2 (44)
	5 yrs.	1.3 (4509)	1.2 (2630)	1.1 (785)	1.3 (517)
Pentad of Fluoridation:		1.2 (8053)	1.2 (5183)	1.2 (1796)	1.1 (991)
After Fluoridation:					
	5 yrs.	1.2 (6971)	1.1 (4340)	1.2 (1986)	1.1 (1142)
	10 yrs.	1.2 (5597)	1.1 (3655)	1.1 (1889)	1.0 (1072)
	15 yrs.	1.0 (2454)	1.0 (1671)	1.1 (1009)	1.0 (633)

Site:		Bone		Other	
Sex:		Men	Women	Men	Women
Prior to Fluoridation:					
	10 yrs.	0.9 (28)	1.1 (13)	1.0 (203)	0.8 (184)
	5 yrs.	1.2 (351)	1.1 (216)	0.9 (1923)	0.9 (2042)
Pentad of Fluoridation:		1.1 (642)	1.0 (438)	1.0 (4436)	1.0 (4884)
After Fluoridation:					
	5 yrs.	1.1 (597)	1.1 (413)	1.0 (4682)	1.0 (4871)
	10 yrs.	1.1 (499)	1.1 (358)	1.1 (5113)	1.1 (5383)
	15 yrs.	1.0 (269)	1.0 (173)	1.2 (3400)	1.2 (3678)

*Number of deaths in parentheses.

(From Hoover, _et al_., 1975)

TABLE V-19

Site and sex specific SMR's and observed number of cancer deaths* (1950-69) in counties in Texas grouped according to natural fluoride levels.

Site	Sex	Control	Low	Intermediate	High
		Levels of Natural Fluoride			
Stomach	Men	1.0 (375)	1.0 (918)	1.1 (239)	1.1 (112)
	Women	1.0 (236)	1.0 (583)	1.0 (122)	1.0 (55)
Kidney	Men	1.1 (121)	1.0 (235)	1.2 (89)	0.6 (19)
	Women	1.0 (68)	1.0 (163)	1.0 (46)	1.2 (23)
Bone	Men	1.0 (40)	1.0 (118)	1.2 (39)	0.6 (8)
	Women	0.9 (27)	1.1 (70)	0.9 (14)	0.8 (5)
Other & Unspecified:	Men	1.0 (319)	1.0 (779)	1.0 (211)	0.9 (83)
	Women	1.0 (318)	1.0 (854)	0.9 (170)	0.9 (73)

*Number of deaths in parentheses.

(from Hoover, 1975).

Moreover, epidemiological studies in England fail to support the hypothesis that stomach or any other cancer is associated with fluoride intake (Kinlen, 1975; Royal College of Physicians, 1976).

An independent evaluation of the data presented by Yiamouyiannis and Burk was carried out by Taves (1976) using the same basic statistics as those used by NCI (U.S. Census and Vital Statistics). To gain more precision, the cancer mortalities observed in the year prior to the census year have been averaged with the figures for the census year.
During evaluation of the data, it was noted that only one of the fluoridated cities had gained in population from 1950 to 1970, whereas seven of the ten nonfluoridated ones had gained. Accordingly, the next ten largest fluoridated cities, seven of which had gained in population over the twenty years, were also evaluated. In addition, data were also compiled on the five large nonfluoridated cities that had been omitted from the original control group.

Results are shown in Figure V-3 and in Table V-20. In no case is there a significant time trend, thus, the assertion that fluoridation has caused an increase in cancer rates does not hold up. The rates in fluoridated cities are higher only for a particular set of cities and the higher rates in these cities were present before fluoridation. When the data for all 20 fluoridated cities and all 15 nonfluoridated cities are combined as shown in Table V-20, the standard mortality ratios for the fluoridated cities are remarkably constant.

For negative results like those described in the preceding section it is important to assess the magnitude of the effect that would escape detection.

TABLE V-20

Standard Mortality Ratios for All Cities, Expected Deaths Based on U.S.A. Rates for 1950

	Non-Fluoridated (15)		Fluoridated (20)	
Time (2 years)	Observed Deaths	S.M.R.	Observed Deaths	S.M.R.
1949-1951	26952	1,1272	53908	1,1961
1959-1961	33377	1,0899	60183	1,1958
1969-1971	38625	1,1390	61938	1,2116

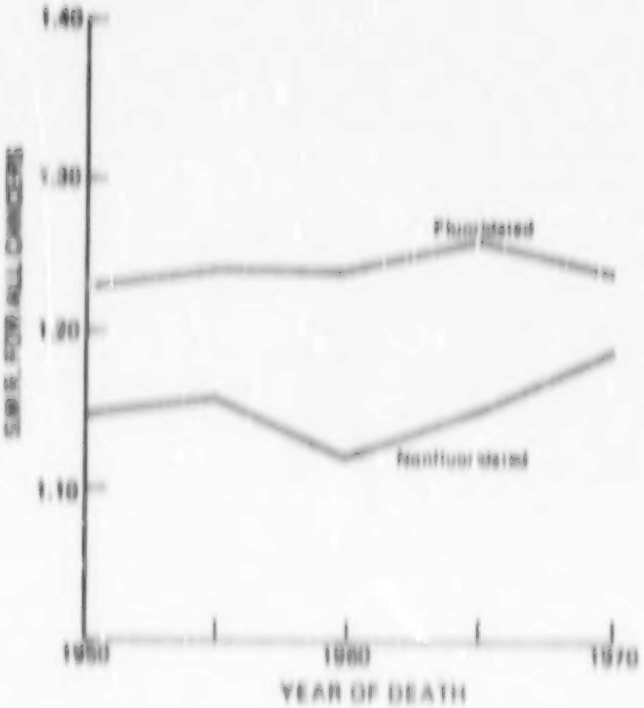

Figure V-2. Standard mortality ratios for all cancers for the cities of Figure VI-1. Reprinted, with permission, from Hoover *et al.*, 1975.

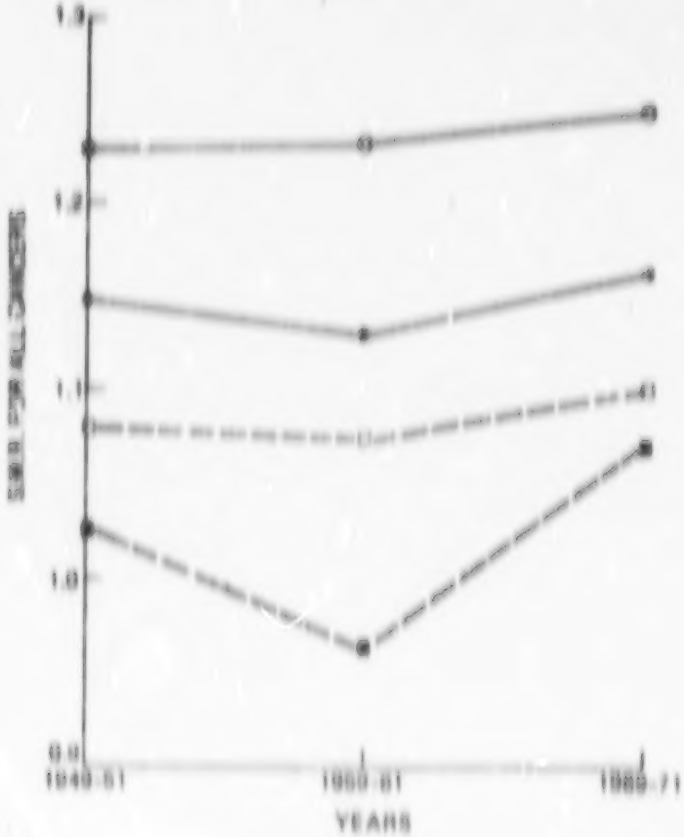

Figure V-3. Standard mortality ratios for all cancers using average of two years observed mortality. Fluoridated cities of Figure V-1, open circles; non-fluoridated cities of Figure V-1, closed circles. Next 10 largest fluoridated cities open squares; the closed squares are the 5 non-fluoridated cities omitted from 15 largest non-fluoridated shown in Figure V-1.

Statistically, such evaluations are known as estimations of error of the second kind, beta error or power of the test (Dixon and Massey, 1969). For the Taves study, for example, it was computed that only 1% increase in cancer death rates would have been detected with 95% confidence. The results of the large NCI study by five-year periods have a similar detection limit with about a 10% detection limit for increase in cancer at specific sites (Taves, 1977).

Other observations of possible positive correlations between fluoride intake and cancer, although not conclusive, deserve attention and further investigation. Okamura and Matsuhisa (1963) showed a correlation between stomach cancer and the fluoride content of rice and "miso". The fluoride values reported for food by Okamura are many times those expected in this country and are based on analytic methods that would not distinguish between organic and inorganic fluoride. So, even if the correlation is causal, it is not clear that fluoride ion is involved. Hirayama (1977) reported that stomach cancer rates in Japan were positively correlated with the amounts of hot tea and fish consumed and negatively correlated with the amount of milk drunk. Tea and fish have been reported to have higher levels of fluoride than other foods, and milk would be expected to act as a binding agent and buffer to reduce effective concentrations of HF in the stomach.

There was an observation in the Kingston-Newburgh (Ast et al., 1956) study which was considered spurious and has never been followed up. There was a 13.5% incidence of cortical defects in bone in the fluoridated community but only 7.5% in the nonfluoridated community. With 474 and 375 children in the respective groups, the t value of 2.65, which is statistically significant (Schlesinger, 1956). Caffey (1955) noted that the age, sex and anatomical distribution of these bone defects are "strikingly" similar to that of osteogenic sarcoma. While progression of cortical defects to malignancies has not been observed clinically, it would be important to have direct evidence that osteogenic sarcoma rates in males under 30 have not increased with fluoridation. The overall bone cancer rates do not appear to have been affected by fluoridation. However, bone cancer rates are dominated by the older age groups, which could obscure a difference in the younger ages.

Taylor and Taylor (1964, 1965) concluded that low concentrations of fluoride increase the rate of tumor growth in animals or eggs. Their studies are not convincing because they show no dose-response effect over a very wide range of doses. Such findings generally indicate inadequate controls or the presence of bias. The use of distilled water for control, rather than an equivalent amount of sodium chloride, might be a cause of inadequacy in the controls. Distilled water tends to pick up metal ions to a greater extent than does a solution. The data of Flemming (1953) suggested a beneficial effect from fluoride at 20 ppm in the drinking water of mice with implanted tumors. An abstract by Bittner and Armstrong (1952) indicated no carcinogenic effect from 5-20 ppm fluoride in the drinking water on the survival of the SBW strain mice.

In summary, the available evidence does not even suggest that fluoridation has increased cancer mortality rates. The margin of possible error is very low, approaching 2/100,000. This is the theoretical effect that could have been missed with present statistical techniques. The NCI studies probably lowered the margin of error by a factor of 5 from the best previous study (Hagen et al., 1954).

Mutagenesis, Teratogenesis, Birth Defects. There are a number of papers suggesting or claiming that fluoride is mutagenic, but relatively little attention has been paid to them. This lack of attention probably stems from the fact that the published evidence has been in plants and Drosophila, primarily with high doses of hydrogen fluoride (HF) rather than fluoride ion. As noted above, the possibility of mutagenesis due to HF is potentially important in cancer of the stomach. Ingested fluoride ion can become HF in the stomach because the pH of HF is 3.18 and the pH of the stomach without food is generally about 1. Although stomach cancer rates show no indication of a relationship to fluoridation in the United States, the much higher stomach cancer rates in Japan are related to intake patterns which are compatible with a hypothesis that fluoride is the crucial factor involved (Taves, 1977). Therefore, the work in plants with HF will be reviewed after consideration of the recent work of Mohamed and Chandler (1976), in which 1 ppm NaF (presumably 0.5 ppm fluoride) reportedly caused permanent damage to the chromosomes of bone marrow cells and spermatocytes in mice. Increased rates of congenital malformations and decreased rates of reproduction can also be consequences of mutation. Hence, the available evidence on these topics as related to fluoride ingestion is also part of this section.

Mohamed and Chandler's experiment consisted of feeding mice a low fluoride diet together with varying concentrations of sodium fluoride in distilled water for two time periods, 3 and 6 weeks. The concentrations of sodium fluoride were 0, 1, 10, 50, 100, and 200 ppm. Sixty-four mice, plus eight baseline controls, were put on a low fluoride diet and given distilled water to drink for one week prior to the start of the experiment. The data consisted of food intake, water consumption, fluoride concentration of the bone ash and cytological examination of chromosomes from bone marrow cells and spermatocytes.

The frequency distribution of chromosomal changes in bone marrow cells was given as a percentage. Two things are of note which are not commented on by the authors. One is the fact that the numbers of cells examined are not consistent with the number of animals in the groups, four to each treatment group and eight to each control group, and vary markedly, 737-1,345 for the groups with the same number of animals. The lack of twice the number of observations in the larger groups raises the question of how animals or slides were selected. Was each animal represented with the same intensity of searching, and if not, what determined the intensity of the search? The second point of note is that the largest change in frequency of abnormality occurs between 0 and 1 ppm NaF. This is odd because there is little or no difference in the bone fluoride concentrations with these two dose levels. A possible difference between the controls and the rest of the animals is that the distilled water dissolved more metal ions than the distilled water with NaF and that this difference in intake was important in the marginally iron-deficient diet (Tao and Suttie, 1976). This type of response makes the claim of an effect down to the 1 ppm level uncertain. Using the 1 ppm NaF animals as the controls, rather than those receiving distilled water, would make the changes in chromosomal fragments unimpressive. There is still, however, a doubling of "ball metaphase" rings and bridges that is recorded. Ball metaphase is not commonly recognized by cytologists and was not noted by Temple and Weinstein (personal communication), when they repeated the work of Mohamed *et al.* (1966a) on onion root tip with 10^{-5} M fluoride.

The spermatocyte changes also show things of note which were not commented on by the authors. In this case, the numbers of cells examined are again very irregular. However, the total for the control groups is approximately double, properly reflecting the group sizes. The serious discrepancy, however, is that there is no way that the percentages given for the frequency of abnormalities can be derived from the number of cells examined. (The cells examined are scored as having either breaks, fragments or both for the first meiotic anaphase and telophase and for the second anaphase and telophase. If each number of cells listed to the left of these two sets of frequencies is divided into 1, 2, 3, etc. to the 4th decimal place, multiplied by 100 and then compared to the percentages listed, only 27 of 90 can be rationalized on the basis of rounding errors. It is, therefore, clear that the listed sets and number of cells, are not the numbers from which the corresponding percentages were derived. It is possible that the authors meant that the number of cells was the sum of two denominators. However, there are several frequencies listed for a given exposure of cells examined would have to have been considerably larger than the total as given.)

Mohamed's earlier work in onion root tips (Mohamed *et al.*, 1966a) and tomatoes (Mohamed *et al.*, 1966b) was done without the use of random numbers to code the slides, but his work in corn (Mohamed, 1970) was done by random assignment of the slide numbers prior to reading, even though this is not mentioned in the paper (phone conversation to D.R. T.) There is apparently no published confirmation of any of this work. An unpublished study (Temple and Weinstein, 1976) was able to confirm increased bridges and fragments of chromosomes in onion root tips when grown in 10^{-5} M fluoride, but they did not confirm the observation of ball metaphase.

The only published second generation study to provide direct evidence of mutation was done by Mohamed (1968). In this study he exposed tomato plants to HF. The summary gives the concentration as 3 mg/m³, but this should read 3 µg/m³ (phone conversation to D.R. T.) The dose or time of parental exposure is not given in the body of the paper. A phone conversation revealed that the seeds came from plants exposed in a previous study (Mohamed *et al.*, 1966b) in which all flower buds past the stage used for the chromosome smears were destroyed so that the fruit grown from the recovered plants would not have been exposed to HF subsequent to the formation of flowers. If the exposure had continued beyond this point, abnormal numbers of cotyledons would not necessarily indicate genetic damage. Abnormal numbers and shapes of the cotyledons occurred in the treated groups about 3 times more often than the 4.7% of the controls. Fasciated petioles, wiry plants and plumuless plants were noted only in the treated plants, but the frequency was irregularly related to the duration of exposure. Dwarf plants and double stalks increased from 0.8% in the controls to 5% in the treated plants with uniform increases in four of the five exposure periods of increasing length. These descriptions of damage sound arbitrary and no criteria are listed, hence, the lack of blind reading leaves the data unconvincing. The article stated that work is in progress to determine whether the effect was due to minute chromosomal changes or to changes within the gene. However, there have been no citations of this article through the June 1976 issue of Citation Index, suggesting that the findings have not been confirmed. Temple and Weinstein (1976) were unable to confirm these findings with the same or a different strain of tomato. The negative results with the same strain are limited however, because of the low rate of seed germination under the conditions used.

There has been work on Drosophila by several different laboratories with a variety of conclusions. Mohamed (1971) claims to have proven that HF in concentrations too low to cause death is mutagenic in Drosophila melanogaster. The basis for his claim is genetic analysis of the progeny of flies exposed for 6 -

12 hours to air which had been bubbled through 2.5% HF. The analysis involved looking at the ratio of the homozygous to heterozygous offspring from F_1 generation sib matings. The tester females were heterozygous for a recessive lethal so that F_2 generations could only be homozygous for the paternal chromosomes in question; i.e., 33% would be expected. These F_1 sibs were selected on the base of marker genes to have the same paternal gene. The control groups showed 34.69 ± 0.86% homozygous and the treated groups showed 26.84 ± 0.09 to 25.52 ± 1.54% homozygous, a clearly significant difference. The author claims a statistically significant difference between the exposed groups, but this is true only if the data in his tables are in error and the ± are standard deviations rather than standard errors, as listed. The small dose effect may be due to having discontinued the introduction of the HF after one hour and erroneously assuming that the concentration stayed constant for the remaining time of the exposure period. In the previous paper (Mohamed and Kemmer, 1970), continuous exposure to air bubbled through 5% HF resulted in only 3 males being left to test which suggests that the use of a 2.5% solution in the 1971 study is quite high. In order to prove the contention of mutagenicity, the males should have been mated prior to treatment to show that their genes were in fact normal before exposure to HF.

Mukherjee and Sobels (1968) reported that injection of 1 mM NaF (unstated amount), as compared to injection of 1 mM NaCl, increased the percent lethals produced by 2000R radiation. The percent lethals in the NaCl groups was about 5% in 4 experiments while it was 6 to 10% in the NaF groups.

Vogel (1973) stated that 1 mM fluoride in 5% glucose fed to Drosophila larva was a weak mutagen but acted as a powerful antimutagen in combination with a strong mutagen. The evidence for a weak mutagen effect is slim. In one of three experiments, there were three lethals compared to one in the controls and one in each of two exposure groups. With only fluoride exposure, the egg laying capacity was clearly depressed in most experiments and the hatchability was generally, but not always lower. Hence, he concluded that fluoride had a sterilizing effect. Treatment with the trialkylating agent Trenimon alone, showed 11.1% lethals but in combination with 12 mM F, 3.4% lethals without a consistent difference in the number of eggs laid or their hatchability. A second experiment showed 6.6 and 1.1%, respectively. Since at least 150 chromosomes were tested for each group in each experiment these differences are clearly statistically significant.

Herskowitz and Norton (1963) showed a marked increase in the incidence of melanotic tumors in two strains of Drosophila. The controls were 0.0% and 7.1%, while the treatment of the larvae with 1 to 10 mM fluoride caused a smooth dose response up to nearly 100% occurrence. The group sizes were 1,500 so the numbers are highly significant statistically.

Obe and Slacik-Erben (1973) reported a 25-50% decrease in the total breaking events of chromosomes of human cells *in vitro* with 3 separate strong mutagens in combination with 1 mM fluoride. Slacik-Erben and Obe's further work (1976) attemping to clarify the role of sodium fluoride and antimutagenic effects with Trenimon gives control data which shows no effect from 1 mM fluoride alone. The human cells in this case were lymphocytes stimulated with phytohaemagglutinin in which nucleotide incorporation and mitotic index were followed for over two days. The data curves for two experiments were averaged and plotted, hence, only a visual judgement of no-effect can be made and no estimate of the power of the test is given.

Jagiello and Ja-Shein (1974) exposed mammalian eggs to NaF and concluded that some changes were taking pace. The earliest effect noted was clumping of the chromosomes at meiosis in cow oocytes exposed to 10 ppm (500 μM). This was the lowest concentration used. There is no indication of blind reading of the smears.

There is some evidence of decreased fertility or reproduction with doses of fluoride below obvious toxic levels, which might be related to mutation and is of interest even if it is not related. Gerdes *et al.* 1971) found a 5-10% reduction in male Drosophila fertility, a 30% reduction of female fecundity and a 20% reduction of egg hatchability from the second generation with six weeks exposure to 2.9 ppm HF. These changes in the second generation (C_1) were attributed to genetic effects rather than to direct effects on the eggs. This claim is not convincing since the eggs giving rise to the first generation would also have been exposed for some hours to the HF.

Rensburg and Vos (1966) concluded that for normal reproduction in Afrikaner heifers under ordinary ranching conditions, the fluoride concentration should be less than 5 ppm. They found that the number of calves produced by the animals on 12 ppm water was decreased by about one half as compared to those drinking 5 ppm. They speculate that there are functional disturbances of the ovaries due to fluoride under range conditions which are marginal for several months each year.

If fluoride were an important mutagen for humans, there would be real concern that cancer rates and congenital malformations might increase. There is a new set of data on each of these possibilities. (See Report of The Royal College of Physicians, 1976, for a review of the largely unpublished earlier data.) In the case of human cancer, the data can rule out an effect larger than 2% (Taves, 1977). Congenital malformation rates, relative to fluoridation, were collected by Erickson et al (1976) in the counties around Atlanta. The overall rates were 292.6 abnormalities per 10,000 live births for the fluoridated and 270 per 10,000 for the nonfluoridated populations. The Chi square was 3.59, and 3.84 would be significant at the 0.05 level. Thus, while the difference is not significant, an increase of 10% certainly can not be ruled out. About half of the population of the county (Fulton County which contains Atlanta) was represented in both the fluoridated and nonfluoridated populations because fluoridation was introduced in the middle of the time when the data were being collected; consequently, the period of exposure was generally short. A second part of this study, however, used data from a ten-year exposure period and shows the opposite relationship; i.e., a statistically significant lower rate in the population exposed to fluoridation. The difficulty in relying too heavily on later data is that the rates are about 1/3 of the Atlanta data, a fact that suggests serious under-reporting. Since a similar degree of apparent under-reporting has been cited to discredit Rapaport's findings on Mongolism (Royal College of Physicians, 1976; Needleman et al., 1975), it is inconsistent to place great faith in this negative study even though it is about 10 times larger than Rapaport's.

The above evidence will not convince most scientists that fluoride is mutagenic for any species, but it certainly does not rule out that possibility, particularly for Drosophila. However, even if fluoride is shown to be mutagenic for species other than man, its relevance to man would be questionable in the face of the available human data.

Mottling. The WHO report (1970, p. 299) includes a table showing the relationship of the community fluorosis (mottling) index (as a function of water fluoride concentrations) to the mean annual temperature. From that table, it was estimated that objectionable fluorosis occurs in the range of 0.8-1.6 mg/liter fluoride, depending on the temperature. No recent U.S. surveys or studies of communities have been found on which a sound decision could be made that greater concentrations are without objectionable effect. A report from Sweden by Ericsson and Ribelius (1971) suggests more mottling at 1.2 ppm than would be acceptable to many. They indicate that only 1 of 92 children had objectionable mottling, but their graph showed 9 graded at 3.0 which was defined as the "whole surface affected, often brown discoloration." They note that this was a higher incidence than previously reported for the same degree of intake. Whether this related to some peculiarity, such as high fish consumption which would not be applicable to U.S. communities is not clear.

Recent work in England has confirmed the view that considerable dental mottling is not due to fluoride and that the optimal concentration of fluoride may, in fact, decrease the amount of dental mottling (Al-Alousi et al., 1975; Jackson et al., 1975). The presence of discolored (brown) areas in a few teeth even in the low-fluoride area suggests that better methods of ascribing etiology need to be developed and the question of objectionable mottling reevaluated.

Two cases of dental mottling in children with diabetes insipidus living in fluoridated communities have been reported (Greenberg et al., 1974). The Royal College of Physicians (1976) report tended to dismiss the possibility that the dental mottling was due to fluoride on the grounds that other conditions might have caused it and that similar observations were lacking in a center for children with renal disease. The fluid intake was estimated to be 2.5-6 times normal; however, it was also stated that the urinary volume was as much as 9 liters/day. Such volume would require an intake 10 times normal (Greenberg et al., 1974). With such unusual intakes, it would not be unreasonable to find dental fluorosis; perhaps the more pertinent question is, "Why is it not noted more often?"

It is possible that objectionable mottling is being avoided in naturally high-fluoride areas in the United States by the practice of drinking bottled water or beverages and by the use of air conditioning which would decrease the fluid intake. Those who do not have these amenities may be either too transient or too nonvocal to be noted. The "moderate" dental fluorosis shown by Hodge and Smith (1965, p. 443) in a community with "about 2 ppm" would be objectionable to most, if not all, parents, although there seems to be little consumer research on the matter.

Recently one of the authors (D.R.T.) visited several areas in Texas (Lubbock and Amarillo) which have high natural fluoride concentrations in their drinking water. Several health officials reviewed for him their observations concerning renal dialysis and dental mottling with respect to fluoride. Their experience has been that there seems to be less bone disease in patients who come to dialysis centers from high fluoride areas. While Lubbock and Amarillo have in recent years decreased the fluoride content of their drinking water supplies, the neighboring towns generally have not changed. The health officials in the surrounding areas have observed a decrease in dental mottling as well as a decrease in rampant caries. In light of these recent observations, it is suggested that such communities be extensively resurveyed before any changes are made in fluoride standards for public water supplies.

In light of the ability of anyone to observe and object to dental mottling, it seems presumptuous for experts to recommend acceptable fluoride concentrations without direct evidence that fluoride at 2 or even 3 ppm is, in fact, causing difficulty.

General Epidemiologic Studies. The largest study of overall mortality rates in high-fluoride (0.7-4.0 ppm) vs. low-fluoride (<0.25 ppm) areas considered 32 paired cities (Hagen et al., 1954). The average mortality in the high-fluoride areas was 1,010.6/100,000, which was 5.6/100,000 higher than in the contol cities. This difference is not statistically significant, because the standard error of the difference is 27/100,000. Conversely, the smallest real difference that the study can be 95% sure of ruling out is 51/100,000, which is 1.69 (the one-tailed critical value for 31 degrees of freedom) times the standard error of the mean plus 5 (the actual difference). In other works, the study would be expected to have this small a difference (or less, only one-twentieth of the times even if there were a real difference of 51/100,000. Considering that the populations studied probably were not very stable, even greater differences cannot be ruled out.

One alternative to increasing the size of the study population is to consider populations exposed to much higher concentrations in the water. The Bartlett-Cameron study (Leone et al., 1954) is an example; fluoride concentrations in the water supplies were 8 and 0.7 mg/liter, respectively. People were selected who had resided for over 15 years in either area but no information was given about whether they drank community or bottled water. The same people were studied ten years later. The authors concluded that there was no statistically significant difference between the two groups taken as a whole. There were however, five of forty tests which showed statistically significant differences, some flavoring the high flouride group. Since several such individual differences would be expected to appear statistically significant by chance with so many tests, these differences do not force rejection of the null hypothesis. The authors of the report conclude that there was not a statistically significant difference between the numbers of deaths. They do not give the age and sex-adjusted values, but these were over twice as high in the high-fluoride area. The AMA committee (1956) that reviewed the data concluded that the difference was just barely statistically significant, 0.045. The AMA committee minimized the importance of this difference by quoting Roholm's statement that he had not observed an increase in mortality among cryolite workers whose fluoride intake was presumably even higher. Roholm, however, also concluded that he could not rule out an increase in mortality. He had only 12 autopsy cases available, and they were not in a controlled study.

It is clear that the studies discussed leave a considerable area of uncertainty. This could be reduced relatively easily. Fluoridation was introduced into many large cities in the early 1950's and into only a few cities since then, so there is a basis for an unusually large study for which the data have been collected but not examined for detrimental or beneficial effects. The positive effects might occur from better nutrition with less caries and tooth loss or from decreased soft tissue calcification. The cancer mortality studies of the NCI involved populations 10 - 50 times larger than those from the 12 paired cities, and the accumulating data will have an even larger potential. The data tapes are all available within the federal agencies and could be run through the same basic programs as set up by the NCI for total mortality, as well as for separate categories of death.

The lack of such studies on the continuing evaluation of fluoridation probably stems from the failure of scientists to ask what the no-effect limits are. Without statements about the power of the tests, the implication of finding no-effect is construed to be that no effect exists. Such implication leaves little incentive to do a better study. Currently, the only motivation for such studies is an interest in checking allegations as they arise. It would be helpful if the NCI were to demonstrate that their study ruled out a much smaller effect than it is possible to exclude on the basis of earlier studies.

Summary and Conclusions. In summary, there is no generally accepted evidence that anyone has been harmed by drinking water with fluoride concentrations considered optimal for the annual mean temperatures in the temperate zones. It seems likely, however, that objectionable dental fluorosis occurred in two children with diabetes incipidus. Bone changes, possibly desirable, have been noted in patients being dialysed against large volumes of fluoridated water. Similar changes can be expected in the rare renal patient with a long history of renal insufficiency and a high fluid intake that includes large amounts of tea. With this particular combination of circumstances, the lowest drinking-water concentration of fluoride associated with symptomatic skeletal fluorosis that has been reported to date is 3 ppm, outside of countries such as India. It should be possible for the medical profession to avoid the possible adverse effects of fluoride under the conditions described above, thereby making it unnecessary to limit the concentrations of fluoride in order to protect these rare patients. On the basis of studies done over 15 years ago, occasional objectionable mottling would be expected to occur in communities in the hotter regions of the United States with water that contains fluoride at 1 ppm or higher and in any community with water that contains fluoride at

2 ppm or higher. However, this may not be the case today; more liberal provisional limits seem appropriate while studies are conducted to clarify the subject.

Research Recommendations

1. Better criteria should be developed for diagnosing dental fluorosis, as distinct from dental mottling.

2. The present rate of dental fluorosis (particularly staining) in communities with fluoride at more than 1 ppm in the water supply should be determined.

3. Bone and blood fluoride concentrations of patients with chronic renal disease in communities with fluoride at 2 ppm or more should be compared with those of similar patients in low-fluoride areas, to see whether there is a difference radiologically, histologically, or clinically, particularly with regard to bone pain and fracture rates.

4. The inorganic fluoride content of food, as distinct from the total fluoride content, should be determined, to settle the issue of whether there has been an increase.

5. Rappaport's study on mongolism should be repeated with the same cities; if there is still an association, intensive case-finding should be carried out, to check whether the lack of case-finding was important in his results.

6. Mortality ratios should be evaluated by cause of death in the fluoridated vs. nonfluoridated areas.

7. There should be *in vivo* studies on the possibility of the development of cellular tolerance or intolerance to fluoride.

8. The nonhuman-primate study of Manocha *et al.* (1975) should be repeated with 5 ppm water and better controls, to check the reported renal enzyme changes.

9. Chromosomal studies of mice drinking water with low F concentrations should be repeated to determine if chromosome abnormalities are induced.

10. Dominant lethal studies should be done in rats and mice by feeding the males various dose levels of fluoride and mating to tester females on a normal diet. This is easier to do and would confirm item 9, if positive, but not necessarily be inconsistent, if negative.

11. Further evaluation of cancer death rates and congenital malformation rates in large fluoridated cities as compared to nonfluoridated cities should be made.

Sodium

Occurrence. Sodium ion is an ubiquitous constituent of natural waters. It is derived geologically from the leaching of surface and underground deposits of salts such as sodium chloride, from the decomposition of sodium aluminum silicates and similar minerals, from the incorporation of evaporated ocean spray particles into rainfall and from the intrusion of sea water into fresh water aquifers. Salt spray from the sea is often the largest contributor of sodium ions within 50-100 miles of seacoasts. Some soils exhibit the property of ion exchange in which calcium ions in the water are replaced by sodium ions during normal leaching.

Human activities also contribute sodium ions to natural waters. The sodium chloride used as a deicing agent on roads enters water supplies in runoff from both roads and storage depots. The quantity of sodium ion from this source has increased progressively since 1947 from about 0.5 million tons to 9 million tons in 1970 (EPA, 1971). This added sodium chloride is distributed throughout the snow belt of the northern U.S. and is most heavily concentrated around metropolitan areas. (Am. Public Works Ass'n., 1969; Hanes *et al*., 1970; Hutchinson, 1971; Bubeck *et al*., 1971).

Municipal use of water typically results in the addition of 20-50 mg/liter of sodium ion, primarily from urine and washing products. Procedures for water treatment often produce a finished water with greater sodium-ion concentration than the raw water from which it was derived. Sources of sodium ion in the treatment of water include sodium hypochlorite, sodium hydroxide, sodium carbonate and sodium silicate.

A survey of 2,100 water supplies, covering approximately 50% of the population of the U.S., was carried out in 1963-1966 by the Heart Disease Control Program, Division of Chronic Diseases and the Water Supply Section, Division of Environmental Engineering, both of the U.S. Public Health Service. The distribution of sodium ion found in this survey ranged from 0.4-1,900 mg/liter as shown in Table V-21. Some 42% of the supplies had sodium ion concentrations in excess of 20 mg/liter and nearly 5% had concentrations greater than 250 mg/liter.

TABLE V-21. Sodium Ion Concentration in Drinking Water

Sodium Ion Concentration mg/liter	No of Samples	Percent of Samples
0-19.9	1,194	58.2
20-49.9	391	19.0
50-99.9	190	9.3
100-249.9	178	8.7
250-399.9	74	3.6
400-499.9	10	0.5
500-999.9	14	0.7
1,000 or higher	2	0.1
Total	2,053	100.1

(From White *et al.*, 1967)

Similar results were shown in the 1975 report of Chemical Analysis of Interstate Carier Water Supply Systems. For 630 systems the range of sodium ion concentrations was from <1 - 402 mg/liter. A total of 42% had concentrations greater than 20 mg/liter and 3% had concentrations greater than 200 mg/liter.

Daily Intake of Sodium Ion. Few studies of habitual sodium ion intake in healthy adults have been reported. Such data as have been reported are based on measurement of sodium excretion in 24 hour or 12 hour urine collections. Since ingested sodium is mostly excreted in urine, these figures give an acceptable estimate of sodium intake for the period immediately preceding and embracing the time urine collection. Wide variations occur among individuals and in the same individual from day to day. (Dawber et al., 1967) Dahl (1958) reported a mean 24 hour excretion of 4,100 mg with a range from 1600-9600 mg in 71 working adult males in New York. Langford et al. (in press) reported a mean sodium excretion of 2822 mg per 24 hours in 171 black women ranging in age from 35-44 years. A recent estimate for infants is 69-92 mg/kg/day (Am. Acad. Pediatrics 1974). Sodium chloride is added to many foods during processing, such as baby foods. Additional sodium chloride is often added during cooking, and again at the table.

Habitual intake of sodium bears no relationship to physiological need. Estimated daily losses of sodium in urine, stool, skin and insensible perspiration in subjects on markedly restricted sodium intake total 40-185 mg/day (Dahl 1958). Additional losses occur during sweating; however, sodium content of sweat is adjusted during sodium deprivation by adrenocortical influences. Healthy individuals have been shown to maintain sodium balance on a sodium intake of less than 2,000 mg per 24 hours while sweating 9 liters per day (Conn, 1949). A variety of preindustrial societies, in widely divergent habitats (tropical jungle, desert, arctic, etc.) subsist for generations on sodium intake less than 1,000 mg/day and show no evidence of sodium deprivation (Dahl 1958; Page, et al., 1974). Total body sodium content is about 130,000 mg for a 70 kg man (Widdowson et al., 1951). Requirements for sodium in growing infants and children are estimated at less than 200 mg/day (Am. Acad. Pediatrics 1974).

It thus appears that habitual intake of sodium in adults in the United States often exceeds body need by tenfold or more. Evidence that this excessive intake may have harmful consequences is summarized elsewhere in this report.

Since adult fluid intake averages 1.5-3 liters/day, sodium intake from drinking water represents less than 10% of the habitual total intake of 3,000-4,000 mg as long as the sodium content of the water does not exceed 200 mg/liter. Adverse health effects may be anticipated with sodium concentrations greater than 20 mg/liter, however, for that special risk group restricted to total sodium intake of 500 mg/day, for intake from food cannot feasibly be reduced to less than about 440 mg/day (White *et al.*, 1967). For this group, whose diets must be medically supervised in a careful manner, knowledge of the sodium ion concentration of the drinking water permits prescription of low sodium water when necessary.

Metabolism. A full description of the physiologic mechanisms governing sodium metabolism is beyond the scope of this report; excellent summaries are available elsewhere (DeWardener, 1973; Early, 1972; Burg, 1976). Sodium is rapidly and almost quantitatively absorbed from the gastrointestinal tract. It is distributed as the most abundant cation of plasma and extracellular fluid in man and all other vertebrates. It is present in bone and (in low concentrations) in cells of most tissues, except adipose tissue, from which it is virtually absent. The concentration of sodium in extracellular fluid is maintained within narrow limits through regulation of the excretion of water by the kidney, under the influence of endocrine, cardiovascular, and autonomic regulatory mechanisms. The total amount of sodium in extracellular fluid thus determines the volume of these fluids. The volume of plasma and of extracellular fluid is in turn governed by regulatory mechanisms involving the endocrine, cardiovascular, and autonomic nervous systems, which regulate volume primarily by influencing renal excretion of sodium. Arterial blood pressure influences and is influenced by variations in plasma volume.

Health Effects

Acute effects. Acute adverse effects of sodium in the healthy population are probably confined to neonates and young infants and will be discussed later.

Chronic effects. An impressive amount of evidence has accumulated over the last several decades that sodium taken in excess of physiologic need is important in inducing an age-related increase in blood pressure that culminates in hypertension in genetically susceptible people.

The prevalence of hypertension in the adult population of United States is 15-20% among Caucasians (U.S. National Center for Health Statistics, 1964) and substantially higher among blacks (Stamler *et al.*, 1976). Thus some 30-40 million Americans are afflicted with this disease.

Evidence both in animals (Dahl *et al.*, 1962) and in man (Ostfield and Paul, 1963; Thomas, 1973) indicates that a genetic factor operates to make some people susceptible to hypertension. One or more environmental factors may then induce the development of hypertension in these people. A wide variety of environmental factors have been implicated in the induction of hypertension, including sodium ion, obesity, trace minerals, and psychosocial stress factors. Evidence that sodium ion is involved as a causative agent in hypertension has three main sources: animal experiments, clinical observations, and epidemiologic studies. The salient features of this evidence are summarized briefly in the following paragraphs.

Animal experiments. Observations (Meneely and Dahl, 1961; Dahl, 1960, 1972) extending over a period of 20 years have repeatedly shown that sodium ion induces hypertension in genetically susceptible rats. Rats given sodium chloride at 2.8-9.8% in diet rations develop hypertension within a few weeks or months. When unselected rats are used in such experiments, the incidence and severity of hypertension vary directly with the sodium chloride concentration in the diet (Meneely and Dahl, 1961). Approximately 80% of animals eventually develop some degree of hypertension elevated blood pressure. (Dahl, 1960). In selective breeding experiments, "sodium-sensitive" and "sodium-resistant strains can be produced by inbreeding separately rats that do and rats that do not develop hypertension in response to sodium chloride (Dahl, 1972). Among genetically sodium-sensitive rats, young animals are more sensitive to the effects of sodium than adults (Meneely and Dahl, 1961). When hypertension becomes established in these animals, it is not corrected by reducing sodium intake (Tobian *et al.*, 1969). Despite many generations of inbreeding, the sodium-sensitive animals will maintain normal blood pressure throughout life if they are never exposed to excessive intake of sodium (Dahl, 1972).

Although there is not general agreement as to the exact mechanism by which excess sodium ion induces hypertension, considerable evidence has accumulated in the last few years that it involves the regulatory mechanisms governing the renal excretion of sodium. Transplantation of kidneys between sodium-resistant and sodium-sensitive rats results in a blood-pressure increase in the rat that receives the sodium-sensitive kidney and a decrease in the rat that receives the sodium-resistant kidney (Dahl *et al.*, 1974).

An important determinant of sodium excretion by the normal kidney is the arterial perfusion pressure (Guyton *et al.*, 1972). Recent studies have shown that the relationship between perfusion pressure and sodium excretion is altered in sodium-sensitive animals in which hypertension has been induced by sodium feeding. Higher arterial pressure is required to excrete the same quantity of sodium in the hypertensive animals than in pair-matched control animals of the same strain whose blood pressure remains normal on high sodium intake (Tobian, 1975).

Correlative data from several other types of experiments supports the importance of sodium excretion as a central determinant of arterial blood pressure in all forms of hypertension, and in normal animals as well.

In recently developed strains of "spontaneously hypertensive rats," (highly inbred animals), hypertension develops spontaneously and does not require induction by sodium ion or other identifiable environmental factors (Okamoto, 1969). Hypertension in these animals is corrected by transplanting into them kidneys from normal animals (Bianchi *et al.*, 1973). From these and other data, it has been suggested that hypertension in the spontaneously hypertensive rat is related to a genetically determined alteration in renal sodium excretion similar to that induced by sodium loading in the sodium-sensitive rat (Coleman *et al.*, 1975).

In animals made hypertensive by constricting one or both renal arteries, hypertension is due primarily to release of renin by the ischemic kidney (Gross, F. 1971). In recent studies of this form of hypertension, a one-kidney model (one kidney constricted, the other excised) and a two-kidney model (one kidney constricted, the other intact) have been used for examining interrelations among blood pressure, sodium ion, and renin (Gavras *et al.*, 1973). Experiments with these models and with inhibitors of the renin-angiotensin system have revealed that, when sodium ion is depleted, arterial blood pressure becomes dependent on the renin-angiotensin system, and that, during sodium loading, the renin-angiotensin system is depressed and arterial pressure depends on sodium ion (i.e., plasma volume). Studies in normotensive animals strongly suggest that this interaction between sodium and the renin-angiotensin system is a primary determinant of blood pressure in normal animals as well (Gavras *et al.*, 1973).

Hypertension may be developed in rats by giving mineralocorticoids, such as desoxycorticosterone (DOC) (Selye *et al.*, 1943). However, if the animals are maintained on a low-sodium diet, hypertension does not develop (Gross, 1960). It appears that the mechanism by which these animals become hypertensive is related to the sodium-retaining action of DOC on the renal tubule.

nephrectomized animals become hypertensive (renoprival hypertension) on ordinary rations. However, if sodium and water intake is restricted, or if plasma volume is maintained by filtration and dialysis, hypertension is prevented or, if established, is corrected and controlled (Braun-Menendez and Covian, 1948).

During the last decade, sophisticated computer models of the circulation have been devised that include all known physiologic control loops. Studies with such models have demonstrated that some alteration in renal excretion of sodium is necessary to produce permanent hypertension (Guyton *et al.*, 1972).

Clinical Studies in Hypertensive Human Subjects. One of the earliest modes of effective treatment for human hypertension was the "rice diet," consisting of rice and fresh fruit and little else (Kempner, 1948). This diet has repeatedly been shown to be effective in lowering blood pressure in approximately 70% of patients with hypertension. The rice diet provides approximately 200 mg of sodium per day. It has been shown that it is the sodium restriction that is responsible for the lowering of blood pressure (Dole *et al.*, 1950; Dahl, 1972). Smaller degrees of sodium restriction do not consistently lower blood pressure in established hypertension (Hatch *et al.*, 1954). Conversely, a very high dietary intake of sodium raises blood pressure in patients with hypertension (Perera and Blood, 1947).

Removal of sodium by regular daily use of diuretic medications is a well-established principle of modern antihypertensive therapy (Page and Sidd, 1973). Use of diuretics alone is effective in significantly reducing blood pressure in 40-66% of patients with hypertension (Conway and Lauwers, 1960; Brest, 1960). A continuing reduction in plasma volume resulting from diuretic therapy occurs in patients who respond to these medications with a reduction in blood pressure (Tarazi *et al.*, 1970) and is thought by many investigators to be responsible for their effectiveness.

In most patients with kidney failure, there is a direct relationship between sodium intake and blood pressure (Ulvila *et al.*, 1972). In such patients, blood pressure is controlled by reducing sodium intake or by reducing plasma and extracellular fluid volume with diuretics, dialysis, or filtration procedures (Vertes *et al.*, 1969; Ulvila *et al.*, 1972). Patients who fail to respond to sodium (volume) depletion can be shown to have renin-dependent hypertension and respond to volume depletion after bilateral nephrectomy (Vertes *et al.*, 1969).

In normal subjects, large loads of sodium do not consistently influence arterial blood pressure in short-term experiments

(Kirkendall *et al.*, 1973). No carefully controlled long-term study has been done to assess the effects of chronic salt loading in normal subjects.

Epidemiologic Studies. Long-term longitudinal studies of U.S. population samples (Kannel *et al.*, 1969, Kannel *et al.*, 1969a; Chiang *et al.*, 1970) have demonstrated beyond doubt that increased blood pressure, of whatever degree, conveys an important risk of cardiovascular disease.

Epidemiologic data bearing on the possibility of preventing hypertension have been reviewed recently (Page, 1976). It is universally agreed that a genetic factor is important in determining susceptibility to hypertension (Ostfeld and Paul, 1963). The exact mode of inheritance has not been clearly established (Thomas, 1973). However, most studies favor the interpretation that a polygenic, rather than single-gene, mode of inheritance best explains the existing data on familial occurrence (Pickering, 1965; Miall and Chinn, 1973; Ostfeld and Paul, 1963).

Both systolic blood pressure and diastolic blood pressure tend to rise with age in the United States (U.S. Center for Health Statistics, 1964) and most other industrialised countries (Hamilton *et al.*, 1954; Miall and Chinn, 1973; Kahn *et al.*, 1972). Although hypertension is most commonly recognised in the fourth and fifth decades of life, recent studies have shown that an upward trend in blood pressure is present in childhood (Zinner *et al.*, 1971, Buck 1973) and that the children of hypertensive parents have higher blood pressure and a stronger upward trend than the children of normotensive parents. These trends are detectable as early as the age of two years (Zinner *et al.*, 1971).

Although long-term prospective studies on the children of hypertensives are still incomplete, available data strongly suggest that early trends in blood pressure presage later expression of hypertension and that efforts to prevent hypertension might best be directed toward infants and young children who are beginning to show age-related upward blood-pressure trends (Buck, 1973).

Population studies in many parts of the world show blood pressure rising with age and prevalences of hypertension comparable with those seen in the United States. Nevertheless, studies of a substantial number of societies that are outside the major Western culture disclose an absence of hypertension and little or no tendency of blood pressure to increase with age. These low-blood-pressure populations represent many different racial groups, climates, customs, and modes of subsistence. When comparisons are made between these populations and people of similar origin who have been assimilated into Western civilization, the acculturated groups show rising blood pressure rising with age. Thus, low-blood-pressure populations are not genetically protected from rising arterial pressure. Explanation must be sought among environmental factors.

Low-blood-pressure populations include Chinese aborigines (Morse and Beh, 1937); Greenland Eskimos (Thomsen, 1927); Melanesian tribes from several areas in New Guinea (Whyte, 1958; Maddocks, 1967) and the Solomon Islands (Page et al., 1974); Polynesians from isolated islands in the Fiji (Maddocks, 1961), Cook (Prior et al., 1968), Caroline (Murrill, 1949), and Tokelau (Prior et al., 1974) groups; Easter Islanders (Cruz-Coke et al., 1964); Australian aborigines (Abbie and Schroder, 1960); nomadic tribes in Kenya (Shaper et al., 1969); Congo pygmies (Mann et al., 1962); bushmen of the Kalahari Desert (Truswell et al., 1972); Masai from Tanzania (Mann et al., 1964); West Malaysians (Burns-Cox, 1970); and, South and Central American Indians from Chile (Cruz-Coke, et al., 1973), Brazil (Lowenstein, 1961), Surinam (Glanville and Geerdink, 1972), and Guatemala (Hoobler et al., 1965). By contrast, rising blood pressure with age and clinical hypertension have been seen in acculturated town-dwelling persons of nearly all these diverse populations.

Acculturation, or the assimilation into the dominant culture of persons from traditionally oriented preindustrial societies, affects nearly every aspect of life. Most often, the multiplicity of forces acting simultaneously makes it impossible to determine the effect of a single factor on biologic changes, such as blood-pressure trends, within populations. Obviously, such factors as general health and nutrition, weight, psychosocial stresses, and the totality of changes involved in acculturation must be considered in interpreting such changes. Low-blood-pressure populations with markedly different diets have been described (Page, 1976). Change toward a more Westernized diet is a universal feature of acculturation. For example, salt, canned meat and fish, rice, wheat, flour, and sugar progressively supplant traditional dietary items (Page, 1976).

In long-term population studies in many countries, weight gain has been the most consistent factor in predicting hypertension (Epstein and Eckoff, 1967; Kahn et al., 1972). The tendency toward rising weight and blood pressure is complex and may be partly genetically linked (Thomas, 1973). Whereas mean weight in industrialized societies, tends to rise with age, this is not true in many preindustrial societies (Page, 1976). However, acculturation is often associated with rising weight, and it is difficult to separate this change from other effects of changed diet and activity.

Among low-blood-pressure populations, low sodium intake has been an invariable feature of habitual dietary patterns (Shaper, 1972; Page, 1976). Where quantitative data are available, sodium intake among low-blood-pressure populations averages less than 2,000 mg/day (Dahl, 1972; Page et al., 1974). Upper limits have not been established. Statements to the contrary have been unsupported by data and appear to be based on an assumption that availability of salt, as in coastal peoples or inhabitants of small islands, is necessarily associated with high sodium intake. The available evidence fails to support this assumption (Page, 1976).

In a detailed analysis of six Solomon Islands societies (Page, et al., 1974), blood pressure was found to be rising with age in females of the three more acculturated populations, but not in the three less acculturated. All six societies were at relatively unacculturated by Western standards, and weight decreased with age in all. Blood-pressure trends were correlated best with sodium intake. The highest blood pressures were found among a group (third out of six in acculturation rank) who had by far the highest sodium intake. A similar relationship between blood pressure and sodium intake has been reported among Polynesians (Prior et al., 1968) and Indians in Brazil (Lowenstein, 1961) and the southwestern United States (Stroto and Short, 1973).

Dahl (1960) reported a rough correlation between sodium intake and prevalence of hypertension among populations. Examples of populations with very high sodium intake and high prevalence of hypertension include northern Honshu, Japan (Dahl, 1960) and coastal fishing villages of Newfoundland (Fodor et al., 1973).

With some exceptions, studies of blood pressure and sodium excretion have failed to show clearcut correlations between these variables in either black or white populations in the United States (Dawber et al., 1967; Langford and Watson, 1975). Lack of significant correlation in intrapopulation studies has been interpreted as evidence against an important etiologic role for sodium in human hypertension. However, the lack of correlation can be equally well explained by the presence of widely variable genetic susceptibility to the effects of sodium on blood pressure plus a high average sodium intake in the population in question. In these circumstances, variation in blood pressure may be related more to the genetic factor than to sodium intake.

In the United States, blood-pressure averages are higher, and the prevalence of hypertension is greater among blacks than among Caucasians (Stamler et al., 1960). In addition, socioeconomic and urban-rural trends have been identified in both whites and blacks with higher arterial pressure in the lower socioeconomic and the more rural populations (Langford *et al.*, 1968). Differences in urinary sodium excretion are not correlated with these trends. However, higher sodium:potassium and sodium:calcium ratios are found in the groups with higher arterial pressure (Langford and Watson, 1971). More research in these areas should be encouraged.

Especially Susceptible Segments of the Population. Data from British studies suggest that a significant number of "crib deaths" (sudden unexpected deaths between the ages of 2 weeks and 2 years) may be due to hypernatremia, and that some infants who do not die may sustain permanent brain damage from hypernatremia. A number of such deaths have been attributed to the use of dry feeding formulas reconstituted with water that is high in sodium. (Robertson, 1975).

Adult Patients Requiring Low-Sodium Diets. The National Center for Health Statistics, on the basis of a representative survey sample of 15,778 subjects aged 12-74 years, estimated that 2.8%, or approximately 6.2 million Americans, are currently on low-sodium diets prescribed for reasons of illness. The low-sodium diets most commonly prescribed limit the patient to either 2.0, 1.0, or 0.5 g of sodium for 24 hours. Where water supplies contain more than 20 mg/liter, dietary sodium restriction to less than 1.0 g/day is difficult to achieve and maintain. White *et al.*, (1967) concluded that 40% of municipal water supplies sampled are unsatisfactory for use with diets that limit sodium strictly.

Analysis. Quantitative analysis of sodium in water poses no serious technologic or methodologic difficulty. Flame emission spectrophotometry is the method for determining sodium ion in solution. It measures the light energy emitted by the sodium ions in aqueous solution after excitation of the sodium atom by passage into a flame. Flame emission spectrophotometry has advantages of simplicity, speed, and sensitivity, with precision comparable with that of the older and more difficult gravimetric method.

Summary and Recommendations. The healthy population includes a large segment (15-20%) who are at risk of developing hypertension. There is evidence linking excessive sodium intake to hypertension, but for man the evidence is largely indirect. The risk of hypertension depends on genetic susceptibility and is influenced by other factors in addition to sodium intake. The development of hypertension is characterized by long latency and slowly rising blood pressure, entering the hypertensive range in middle life. People at greatest risk cannot be identified with certainty in advance. For most people, the contribution of drinking water to sodium intake is small in relation to total dietary intake.

Because drinking water is an obligatory dietary ingredient, concentrations should be maintained at the lowest practicable levels, and trends toward increasing concentrations of sodium in water supplies as a result of deicing and water-softening procedures should be discouraged. Optimal concentrations of sodium should be regarded as the lowest feasible.

Specification of a "no-observed-adverse-health-effect" level in water for a substance like sodium for which the effect is associated with total dietary intake and for which usual food intake is already greater than a desirable level is impossible.

The defining of upper allowable limits is inevitably arbitrary. Reduction in hypertension for a small segment of the U.S. population who are on severely restricted diets requires a total intake of sodium less than 500 mg/day. These persons need water containing less than 20 mg/liter sodium ion.

A larger proportion of the population, about 3%, is on sodium-restricted diets calling for sodium intake of less than 2,000 mg/day. The fraction of this that can be allocated to water varies, depending on medical judgment for individual instances. Knowledge of the sodium ion content of the water supply and maintenance of it at the lowest practicable concentration is clearly helpful in arranging diets with suitable sodium intake.

It appears that at least 40% of the total population would benefit if total sodium ion intake were maintained not greater than 2,000 mg/day. With sodium ion concentration in the water supply not more than 100 mg/liter, the contribution of water to the desired total intake of sodium would be 10% or less for a daily consumption of two liters.

Research Needs

1. A large and impressive body of data has been accumulated that relates excessive sodium intake to the development of hypertension. Nevertheless, the role of sodium in hypertension remains controversial. Genetic factors, hormones, other dietary factors, and psychosocial stresses also influence blood pressure in important ways. Research should be encouraged to clarify the relative roles and interactions of these influences and the mechanisms by which blood pressure is affected at the physiologic and cellular levels.

1. More information is needed on the average daily intake of sodium, potassium, calcium, and trace metals by different

segments of the U.S. population and on the relative contributions of water and other dietary sources to intake.

3. Also more information is needed on day-to-day and seasonal variations in the composition of water supplies and on the variation in water intake in different segments of the population.

4. Removal of sodium from water by methods currently available is expensive and inefficient. Research directed to developing efficient methods for bulk desalinization of water should be encouraged.

5. Use of sodium chloride for deicing roads results in continuously increasing the sodium content of public water supplies. Research should be continued toward alternative methods of highway ice control.

Nitrate

Occurrence. Nitrate ion is the thermodynamically stable form of combined nitrogen for terrestrial, oxygenated aqueous systems. Accordingly, there is a tendency for all nitrogenous materials in natural waters to be converted into nitrate. All sources of combined nitrogen must, therefore, be regarded as potential sources of nitrate.

Major point sources of combined nitrogen are municipal and industrial wastewaters, refuse dumps, animal feed lots and septic tanks. Diffuse sources include runoff or leachate from manured or fertilized agricultural lands, urban drainage and biochemical nitrogen fixation. Some tenths of a milligram per liter of combined nitrogen occurs in rainfall from solution of atmospheric ammonia and oxides of nitrogen.

In the Community Water Supply Survey of the Bureau of Water Hygiene in 1969, the range of nitrate concentrations found was 0.0-127 mg/liter. Nineteen systems, about 3% of those examined for nitrate, had concentrations in excess of the then recommended limit of 45 mg/liter.

Ground waters from shallow wells often have large concentrations of nitrate. Statewide records in Illinois (Table V-22) show high nitrate to be more common in wells less than 50 feet deep (Larson and Henley, 1966).

TABLE V-22

Well Depth and Nitrate Content of Water

		Analyses with Nitrate (as Nitrogen) Higher than Concentration			
Depth Well m	No. Analyses	0.2 mg/liter	3 mg/liter	10 mg/liter	20 mg/liter
0-8	480	87%	56%	28%	13%
9-15	926	80%	40%	20%	10%
16-30	1,568	64%	18%	5%	1.8%
31-60	2,082	61%	11%	3%	0.7%
over 60	3,828	55%	5%	0.6%	0.1%

(from Larson and Henley, 1966)

(Larson and Henley, 1966). For example, 81 percent of 221 dug wells and 34 percent of drilled wells in Washington County Illinois contained more than 10 mg/liter of nitrate-N. Farm ponds had less than 3 mg/liter nitrate-N. (Dickey, *et al.*, 1972). Analyses of over 5,000 waters in Missouri (8) showed that 27 percent of the waters contained nitrate-N in excess of 10 mg/liter. (Smith, 1970). Concentrations of nitrate in farm ponds were much less, similar to those found in Illinois. Forty-five percent of 250 wells in Wisconsin that were examined twice monthly for more than a year consistently yielded water containing more than 10 mg/liter nitrate-N and 71 percent of the well waters exceeded this level at least once. (Crabtree, 1970).

In Nassau County, N.Y. 370 wells supply 1.5 million people. In 1969, water from twenty of these wells showed more than 10 mg/liter nitrate-N. (Smith and Baier, 1969). In Southern California, certain public water supplies have exceeded 10 mg/liter nitrate-N since 1935.

In Hall County, Nebraska (Piskin, 1973) nitrate concentrations in well-water samples were related to characteristics of the unsaturated zone.
Hydraulic conductivity of the unsaturated zone permitted excessive water and nitrate of the surface to reach the aquifer at 10-100 feet depth (Table V-23).

TABLE V-23

Nitrate Concentration in Water Samples and
Sand Percentage in the Unsaturated Zone in Hall County, Nebraska

Fraction of Sand in Unsaturated Zone %	Nitrate concentration, mg/liter					
	<10		≥10		≥45	
	No.	%	No.	%	No.	%
< 25	63	77.8	18	22.2	7	8.6
25 - 50	45	73.8	16	26.2	1	1.6
50 - 75	69	47.6	76	52.4	3	15.9
> 75	99	44.2	125	55.8	34	15.2
Total wells	276	--	235	--	65	--

(from Piskin, 1973)

Observations on fertilised soils in Illinois showed no signs of nitrate percolation to groundwater. No observations were made on sandy soils.

The U.S. Geological Survey has records on nitrate concentrations in six major rivers from 1959 to 1970. Trends toward increasing concentration were indicated for the Delaware, San Joaquin, and Ohio rivers. No distinct trend was observed in the Colorado river and trends to decreased values were noted for the Missouri at Nebraska City and the Brazos river. Overall concentrations ranged from 0.6-1.5 mg/liter nitrate-N.

Analyses by the Illinois State Water Survey since 1945 show trends toward increased nitrate for the Wabash, Ohio and Mississippi rivers (Harmeson, *et al.*, 1973). For other streams within Illinois the trends to greater concentrations were sharper than those of the major rivers. Characteristically, the highest levels occurred during the spring rainy season in the smallest watersheds, where intensive cultivation of row crops necessitated high fertilisation and tile drainage (Harmeson, *et al.*, 1971). In 1897-1899, the load from the Illinois River at Kampsville was calculated to be 2.33 kg nitrate--nitrogen/hectare/y, and in 1900-1902, the load was still 2.33 kg/hectare/y (Palmer, 1903). In 1956-1961, at nearby Peoria, the load was 8.6 kg/hectare/y; in 1961-1966, it was 13.6 kg/hectare/y; and in 1966-1971, it was 16.7 kg/hectare/y (Harmeson, *et al.*, 1973).

When downstream concentrations in surface waters are examined, it must be realised that nitrate levels may decrease by dilution, and by rapid assimilation of nitrate into aquatic plants. Also, when trends are examined it would be recognised that the rate of application of nitrogenous fertilisers increased only gradually from 1945-1960, but increased five-fold from 1960-1967 and then leveled off with only a gradual increase to the present.

Nitrate may be biochemically assimilated from water by growing plants or may be converted to gaseous nitrogen in anoxic situation. In oxygenated waters, it is quite stable and not easy to remove. Denitrification to nitrogen gas and ion exchange are technically feasible processes for nitrate removal, but they have not been extensively developed on a municipal scale. So, concentrations in finished drinking waters at the tap are usually the same as those in the source waters.

Other Sources of Nitrate. Ordinarily, the major human intake of nitrate is from food rather than from water. The mean food intake for nitrate plus nitrite in the United States has been estimated to be nearly 120 mg/day (White, 1975) most of it coming from vegetables such as celery, potatoes, lettuce, melons, cabbage, spinach and root vegetables, some of which may contain several thousand parts per million of nitrate (Table V-24).

Table V-24

Estimated Average Daily Ingestion for United States Resident†

SOURCE	Nitrate mg	Nitrate %	Nitrite mg	Nitrite %
Vegetables	86.1	86.3	0.20	1.8
Fruits, juices	1.4	1.4	0.00	0.0
Milk and products	0.2	0.2	0.00	0.0
Bread	2.0	2.0	0.02	0.2
Water	0.7	0.7	0.0	0.0
Cured meats	9.4	9.4	2.38	21.2
Saliva	30.0*		8.62	76.8
Total	99.8	100	11.22	100

*Not included in total.

†From J. White, Jr., J. Agr. Food Chem. 23:886 (1975).

Nitrate is secreted in the saliva, the mean value being about 40 mg/day, of which about 10 mg/day is reduced to nitrite and found in that form. These quantities, although internally derived, also represent inputs to the gastric system.

The variability from individual to individual must be very high, because different classes of foods vary significantly in nitrate content from near zero for fruits, milk, and cereal products to thousands of parts per million for certain vegetables. Aside from vegetables, the next largest contribution comes from cured meats. The history of use of nitrate and nitrite for meat-curing has recently been reviewed (Binkerd and Kolari, 1975). The amounts of nitrate and nitrite used in meat-curing have been on the decline for many years, and regulations expected to be promulgated in the near future will decrease their use even further. Thus, it is likely that vegetables will continue to supply the bulk of dietary nitrate.

Man's exposure to nitrite is also summarized in Table V-24. Most is a result of reduction of nitrate in saliva (Tannenbaum et al., 1974) to nitrite. However, other routes of reduction may occur.

Metabolism. Little is known of nitrate metabolism in man. It is generally assumed that absorption takes place in the upper portion of the small intestine and that excretion is primarily, if not exclusively, through the kidney (Soliman, 1957).

It is well known that nitrate is absorbed in the upper gastrointestinal tract (Soliman, 1957) and concentrated from the plasma into saliva by the salivary glands (Burgen and Emmelin, 1961). Although the rate of clearance of salivary constituents is highly variable, the cleansing of mucosal surfaces is quite efficient, and clearance is rapid (Gibbons and van Houte, 1975). A great deal of effort has gone into the study of iodide distribution in man, but very little into the study of nitrate distribution. However, Burgen and Emmelin (1961) summarized a number of studies which suggested that nitrate is transported by the same ion-transport system as iodide, perchlorate, and thiocyanate. Furthermore, the transport system operates in the thyroid, gastric mucosa, and mammary glands, as well as in the salivary ducts.

It is of considerable significance that major differences occur among mammalian species in their ability to concentrate nitrate from plasma into saliva (Cohen and Myant, 1959). Large inter-species differences have also been shown to occur in the elimination kinetics of nitrate (Schneider and Yeary, 1975). Preliminary observations on a number of species have shown that not all animals reduce nitrate to nitrite in saliva (P. M.

Newberne, personal communication). Thus, nitrate metabolism in man cannot be readily predicted from animal data.

Several studies have suggested that large differences in nitrate metabolism may occur between individuals. These differences can span about three orders of magnitude when all the available data, including diet and physiological status, are taken into consideration.

Earlier investigations did not consider the effects of conversion of nitrate to nitrite in saliva. Recent studies (Tannenbaum, in press) have demonstrated that high nitrate intake can lead to large increases in the concentration of salivary nitrite. Although the pattern and extent of nitrite concentration vary from individual to individual, all persons tested had very large increases in nitrite concentration after consumption of even a small quantity (50 ml) of celery juice containing sodium nitrate at 1,200 µg/liter.

Salivary nitrite concentrations observed after consumption of celery juice are different from those reported by investigators using different foods as nitrate sources. Harada *et al.*, (1975) found nitrate at 600 µg/liter or higher in saliva after ingestion of salted Chinese cabbage. The nitrite observed under these conditions reached about 100 µg/liter. These studies were performed on only a few people. Stephany and Schuller (1974) fed people several vegetables under a variety of conditions and observed increases in nitrite. In one experiment, a cooked onion-like vegetable (postelein) caused an increase in nitrite to about 200 µg/liter in 2 hours; it reached 300 µg/liter in 17 hours and declined slowly thereafter. In the work of Spiegelhaider *et al.* (1970), beet juice was the source of nitrate, and maximum nitrite concentration of about 150 µg/liter was observed.

There does not appear to be any significant discrepancy among these observations. Two major factors other than nitrate intake determine the salivary nitrite content: the subject's oral microflora and other constituents in the nitrate-containing food.

It is not yet possible to construct a complete pharmacokinetic model of nitrate metabolism, but such a model will have to incorporate the following salient points (Tannenbaum *et al.*, in press):

1. The half-life for clearance of nitrate, and consequently nitrite, is about 12 hours.
2. The maximal concentration of nitrite in saliva depends primarily on nitrate intake up to approximately 100 mg of sodium

nitrate and increases relatively little with intake greater than 100 mg.

3. The nitrate source is as important in determining salivary nitrite concentration as the amount of nitrate consumed.

4. The conversion of nitrate to nitrite depends on the oral microflora.

Health Aspects. Two health hazards are related to the consumption of water containing large concentrations of nitrate (or nitrite): induction of methemoglobinemia, particularly in infants, and potential formation of carcinogenic nitrosamines.

Acute toxicity of nitrate occurs as a result of reduction to nitrite, a process that can occur under specific conditions in the stomach, as well as in the saliva. Nitrite acts in the blood to oxidize the hemoglobin to methemoglobin, which does not perform as an oxygen carrier to the tissues. Consequently, anoxia and death may ensue.

Healthy adults are reported to be able to consume large quantities of nitrate in drinking water with relatively little, if any, effect (Bosch et al., 1950; Gregeron et al. Acute nitrate toxicity is almost always seen in infants rather than adults. This increased susceptibility of infants has been attributed to high intake per unit weight, to the presence of nitrate-reducing bacteria in the upper gastrointestinal tract, to the condition of the mucosa and to greater ease of oxidation of fetal hemoglobin (Walton, 1951).

Gastric pH greater than 4 is conducive to the growth of nitrate-reducing bacteria. Such gastric conditions are likely to occur with infants, who are prone to upset stomachs and achlorhydria (Comly, 1945).

Assessment of maximum nitrate levels in water exhibiting no adverse health effects has been based principally on a study of known cases of methemoglobinemia. The early survey of Walton showed that no cases on methemoglobinemia were reported when the water contained less than 10 mg/liter nitrate as nitrogen. Later, Hatteimacher (1962) found 3% of 467 cases in which the nitrate concentration of the water supply was less than 9 mg/liter as nitrogen and Simon et al., (1964) found 4.4% of 249 cases in which the recorded concentration of nitrate as nitrogen was less than 11 mg/liter.

Acceptance of these results as definitive is complicated by several factors:

1. Nitrate analyses frequenly were performed some time after the case had occurred and so the concentration at the time of the illness is not really known.

2. Boiling of the water prior to feeding would have concentrated the nitrate over that in the raw water.

3. Incidence of toxicity should be related to total intake of nitrate, not just water concentration. Concurrent ingestion of vegetable puree or juice could have provided much enhanced nitrate intake in some cases.

4. Only one of the U.S. cases has been associated with a public water supply, regardless of nitrate content. It is known that many infants have drunk water in which nitrate as N was greater than 10 mg/liter without developing methemoglobinemia.

Studies supplementary to the previous ones in which levels of methemoglobin in the blood of infants were related to concentrations of nitrate in the water being fed have been reported by Winton (1971) and by Shuval and Gruener (1975). Both studies showed detectable enhancement of methemoglobin levels in infants being supplied with water containing nitrate as nitrogen only slightly in excess of 10 mg/liter. Although the full significance to health of increases in sub-clinical levels of metheroglobin is unclear, they presumably represent an onset of the toxicity leading to methemoglobinemia and therefore refer to a maximum level of nitrate for no-adverse-health effects.

Normally less than 2% of total hemoglobin is present as methemoglobin (Ferrant, 1946; Gross, 1964; Jaffe and Heller, 1964). No external signs or symptoms are noted generally as long as the methemoglobin is less than 5%. Between 5 and 10% methemoglobin the first signs of cyanosis can be seen (Knotek and Schmidt, 1964). In trained subjects undergoing work tests, 10-20% methemoglobin was found to result in impaired oxygenation of muscles. A Russian study of 800 children in day nurseries (Subbotin, 1961) found that over 90% of them who ingested water with 20-40 mg/liter of nitrate had elevated levels of blood methemoglobin and 50% showed levels in excess of 5%. On the other hand, there was no elevation of methemoglobin level with 9 mg/liter of nitrate in the water.

It can be concluded that, from the view-point of induction of methemoglobinemia, the maximum concentration of nitrate in water exhibiting no-observed-adverse-health effect is close to the interim standard of 10 mg/liter as nitrogen. However, there appears to be little margin of safety for some infants with the standard at this concentration.

There appear to be five possible conditions for poisoning of man or animals:

1. The presence of microorganisms in the rumen of cattle causes reduction of nitrate to nitrite. Absorption of the nitrite ion can result in toxicity to cattle (Bradley *et al.*, 1939). The enlarged cecum and colon of horses also provide a location for microbial reduction of nitrate (Bradley *et al.*, 1940; Knotek and Schmidt, 1964).

2. The more alkaline stomach of infants younger than 4 months old allows growth of microorganisms that can reduce nitrate to nitrite; therefore, high-nitrate water can be toxic to the very young (Knotek and Schmidt 1964; Richards and Knowles 1969; Schuphan 1965).

3. When processed or unprocessed spinach is stored under conditions permitting growth of microorganisms, nitrate can be reduced to nitrite. Spinach left at room temperature for some time after cooking or after a jar of baby food has been opened has caused toxicity in babies. Other vegetables or prepared foods high in nitrate can also cause problems (Schuphan 1965; Simon, 1966; Sinios and Wodsak, 1965).

4. Nitrite has occurred in damp forage materais that were high in nitrate. Ingestion by livestock proved toxic (Olson and Moxon, 1942).

5. There have been cases of outright nitrite poisoning where the legal limit for food additives has been exceeded by a factor of 10 or where nitrite has been mistaken for common salt.

A summary of studies on direct acute toxic effects of ingestion of nitrate and nitrite by different species of animals, including man, has recently been published (Ridder and Oehme, 1974). Excerpts from their report are presented in the following paragraphs:

> "Monogastric animals are more tolerant to excessive levels of nitrate in their diet than are ruminants. Dogs have been fed up to 2% (20,000 ppm) nitrate in their diet without any adverse health effects (Olson *et al.*, 1972; PHS, 1962). Rats have been fed approximately 1% (10,000 ppm) nitrate for a lifetime without adverse effects (PHS, 1962). Healthy human adults are reported to be able to consume large quantities of nitrate in drinking water with relatively little, if any, effects (Bosch *et al.*, 1950; Oregeron *et al.*, 1957).
>
> However, the physiological effects noted with ingestion of nitrate in food and water are variable. This may be explained by the metabolic and physiologic differences existing between animals. Toxicity usually results when nitrate in the food is reduced to nitrite prior to ingestion. Under specific conditions, this reduction can occur in the stomach. Some tissues of all animals are able to reduce nitrate to nitrite.

In swine, larger doses of nitrites are required to produce the methemoglobin levels necessary for acute toxicity (Curtin and London, 1966). The pig was found to have the slowest rates of methemoglobin formation and methemoglobin reduction (Smith and Beutler, 1966). A relative lack in response to treatment with methylene blue was also noted.

"Swine are only susceptible to nitrite when the nitrite is pre-formed (Blood and Henderson, 1960). The toxic level of nitrites for pigs is listed as 88 mg/kg.

"Some of the clinical signs of chronic nitrate toxicity in swine are vitamin A deficiency, thyroid dysfunction, decreased rate of gain, arthritic conditions, and abortions. Lymphocytic leukocytosis and erythrocytosis have also been reported (Curtin and London, 1966).

"Nitrate, when given orally to dogs was reduced to nitrite, but the amount reduced varied from practically none to a quantity sufficient to bring about methemoglobinemia (Singer, 1968). When given orally or intravenously, the nitrate ion caused over-excretion of chloride, resulting in hypochloremia, alkalosis, and digestive disturbances (Green and Hiatt, 1954). Dehydration occurred due to the diuretic effect of nitrate (Green and Hiatt, 1955).

"The physiologic effects of nitrate toxicity in humans are largely unknown. Acute nitrate toxicity is almost always seen in infants rather than adults, and results from ingestion of well waters and vegetables high in nitrates. Comly (1945) deduced that infants were prone to upset stomachs and achlorhydria. As a result, the stomach pH increased in alkalinity allowing nitrate-reducing organisms to enter and to reduce nitrates to nitrites. A gastric pH above 4 supports nitrate-reducing organisms (Bosch *et al.*, 1950; Amer. Acad. Ped. Comm. Nut., 1970). Digestive disorders causing injury to the gastrointestinal mucosa and the resulting increased absorption was evident in several cases of infant methemoglobinemia reported in Minnesota (Bosch *et al.*, 1950).

"Immature enzyme systems may also be of importance (Comly, 1945). Methemoglobin formation rate in human adults is close to the rate observed in cattle. However, in man, the rate of methemoglobin reduction occurred the fastest of all species studied (Smith and Beutler, 1966). Approximately 1% of the adult hemoglobin is present as methemoglobin (Jaffe and Heller, 1964). This constant concentration exists as a result of the balance between the oxidation of hemoglobin and the reduction of methemoglobin. Fetal hemoglobin (hemoglobin F) is oxidized by nitrite to methemoglobin at a rate twice as rapid as adult hemoglobin (hemoglobin A). Furthermore, the enzymatic capacity of the erythrocytes of newborn infants to reduce methemoglobin to hemoglobin appears less than that of adults (Jaffe and Heller, 1964). The difference is probably due to a developmental deficiency in the activity of DPNH-methemoglobin reductase (diphosphopyridine nucleotide) (Jaffe and Heller, 1964). As opposed to adults, several clinical, physiologic, and metabolic factors predispose infants to development of methemoglobinemia and acute nitrate poisoning."

Several studies have been conducted on long-term chronic feeding of nitrite to rats. In one study (Druckrey *et al.*, 1963), rats received sodium nitrite at 100 mg/kg in drinking water daily during their entire life span over three generations; no evidence of chronic toxicity, carcinogenicity, or teratogenicity was found. A second investigation (Greenblatt *et al.*, 1973) involved feeding sodium nitrite alone or with amino acids to rats for 67 weeks, for a total dose of 3.35 g of sodium nitrite per rat; no effect was seen in any of the animals receiving nitrite. A third study (Van Logten *et al.*, 1972) involved feeding rats canned meat processed with sodium nitrite at 5,000 ppm over a lifetime; no effect was seen in any of a large variety of chemical, biochemical or histopathologic indexes in the test animals.

However, in other laboratory studies (Shuval and Gruener, 1972) chronic exposure of rats to sodium nitrite at 2,000 and 3,000 mg/liter in drinking water for 2 years was associated with distinct pathologic changes in heart and lung tissues. Also, mice chronically exposed to sodium nitrite at 1,000 and 2,000 mg/liter in drinking water showed reduced motor activity. EEG recordings from implanted electrodes revealed major changes in brain electric activity in rats receiving nitrite at 100 - 2,000 mg/liter. These changes persisted after exposure to nitrite ceased.

Gruener *et al.* (1973) showed that transplacental passage of nitrite occurred in pregnant rats given doses at 2.5-50 mg/kg orally or interperitoneally with production of methemoglobinemia in the fetuses. The concentrations of nitrite and methemoglobin reached in the fetal blood were lower than those in the maternal blood. Fetal rats were found to have methemoglobin reductase activity approximately 10 times higher than that of adult rats. In contrast, human adult blood exhibited methemoglobin reductase activity 1.5 times higher than did human cord blood (Gruener *et al.*, 1973). Rats born of dams exposed to nitrite during gestation had high mortality rates and poor growth and development.

The other health hazard proposed for nitrate in water, that it may act as a pro-carcinogen, is much more speculative. A series of reactions is involved by which it is proposed that nitrate in water may be converted to N-nitroso compounds that are the direct carcinogenic agent. The steps in the reaction sequency are:

1. reduction of nitrate to nitrite.
2. reaction of nitrite with secondary amines or amides in food or water to form N-nitroso compounds.
3. carcinogenic reaction of N-nitroso compound.

To the extent that this series of processes actually operates in the human body, nitrate has a capacity to become a pro-carcinogen. In this event, potential carcinogenesis will be the major hazard involved in the ingestion of nitrate. The full series of reactions has not yet been demonstrated, however, so that the problem is a prospective rather than a realized one. The possible role of nitrate in water in contrast to the role of the normally much greater ingestion in foods has also not been determined.

More than one hundred N-nitroso compounds have been tested for carcinogenicity and about 75-80% have been found to cause cancer in animals (Magee and Barnes, 1967; Druckrey *et al.* 1967; Shank 1975; Mirvish, 1975a). Although there is no definite evidence that nitrosamines or other N-nitroso compounds have induced cancer in man, several suggestive epidemiological correlations have been reported (Clifford, 1970; Fong and Chan, 1973; Burrell *et al.*, 1966; Mirvish, 1972; Gregor, 1974) and there is no reason to suppose that man is not susceptible.

Reaction of nitrites and secondary amines or amides occurs readily in acidic solution and particularly at the normal pH range of 1-5 characteristic of gastric contents after a meal (Mirvish 1975a, 1975b). Moreover, simultaneous feeding of nitrite and amines to mammals results in the formation of nitrosamines in the stomach and the producton of gastric tumors (Sander *et al.*, 1968; Greenblatt *et al.*, 1971; Sander and Schweinsberg, 1972; Mirvish, 1975b; Newberne and Shank, 1973). On the other hand, cancers were not observed when nitrate and amine were administered together to mice, indicating little reduction of nitrate to nitrite in the mouse stomach.

The relation of nitrate concentrations in water supplies to the first step in the reaction sequence for man [illegible] still more problematic, however. The major source of nitrite to the stomach, at least for healthy individuals, is the saliva, normally containing 6-15 mg/liter of nitrite (Tannenbaum *et al.*, 1974). The ingestion of foods, such as vegetables or vegetable juices containing nitrate at hundreds or thousands of milligrams per liter, can lead to much geater salivary concentrations of nitrite, in the range of hundreds of milligrams per liter (Tannenbaum, in press). However, the effect of concentration of nitrate in drinking water on salivary nitrite concentration is not known.

Nitrite may be formed from nitrate in the stomach by bacterial reduction, as discussed in connection with the induction of methemoglobinemia. Little reduction occurs in man, however, unless the gastric pH is greater than 5.0 (Mucha *et al.*, 1965; Sander and Schweinsburg, 1968). Thus, the pH condition for formation of nitrite is quite different from the pH range for ready formation of N-nitroso compounds, pH 3.5 or less.

Other organs of interest in connection with nitrate reduction and N-nitroso compound formation are the infected urinary bladder, the large intestine and the mouth itself (Mirvish, 1975b). Brooks *et al.* (1972), for example, found dimethylnitrosamine in the urine of two people having *Proteus mirabilis* infections of the bladder.

Epidemiologically, correlations have been shown to occur between incidence of gastric cancer and concentration of nitrate in the drinking water. For example, Hill *et al.* (1973) pointed out that the town of Worksop, England, with 90 mg/liter nitrate in the drinking water for many years, had an incidence of gastric cancer 25% greater (100% greater for those 75 and over) than that of similar control towns. In the control towns, the weekly nitrate intake was about 400 mg (100 mg from meat, 200 mg from vegetables, 100 mg from water) whereas in Workshop the total intake was 900 mg/week, with 600 mg from water.

Similarly, it has been shown that the unusually high incidence of stomach cancer in certain mountainous areas of Colombia (Hawksworth *et al.*, 1975; Correa *et al.*, 1975) is associated with high concentration of nitrate in the drinking water. In the area of high cancer incidence, there is 110 mg/liter nitrate in the drinking water compared to much lower values in control low-incidence areas. As much as 180 mg/liter nitrate was found in the urine of persons in the high-incidence area, but never more than 45 mg/liter in the low-incidence regions.

Findings such as these are preliminary and suggestive. They provide no firm evidence of a causal link between incidence of cancer and high intake of nitrate. They do indicate a need for caution in assessment of lack of adverse health effects even at the 10 mg/liter concentration level for nitrate as nitrogen and a need for continued intensive study on the metabolism and effects of nitrate in man.

Analysis. A variety of methods for determination of nitrate exists, but none is particularly precise, accurate or sensitive in the milligram per liter concentration range (Schuller and Veen, 1967; Boltz, 1973). Further development and standardization of analytical methodology will be required if standard routine determinations are to be considered reliable within the range required for proper control and assessment of health effects.

Most standard procedures for nitrate determination in the mg per liter range are spectrophotometric. Traditionally, three types of reaction of nitrate have been used as bases: nitration of a phenolic substance to a colored derivative; oxidation of an organic substance to a colored product; and reduction of the nitrate to nitrite or ammonia, followed by reaction of the reduced nitrogenous materials to give colored substances. In addition, direct spectrophotometric determination based on ultraviolet absorption of nitrate at 273 nm is possible and becoming established. Electrochemical determination with the use of a nitrate electrode may also be feasible, but is subject to numerous interferences. (Usher and Telling, 1975; Milham *et al.*, 1970; Morie *et al.*, 1972).

Substrate reagents used in the nitration techniques include: phenoldisulfonic acid, salicyclic acid, 4-methyl umbelliferone, 2, 4-xylenol, 2, 6-xylenol, 3, 4-xylenol and toluene (Usher and Telling, 1975).

Reagents that have been used for oxidation reactions are brucine, diphenylamine, diphenylbenzidine, diphenylamine-sulfonic acid, strychnine and strychnidine (Usher and Telling, 1975).

With all these techniques there has been a consistent problem of reproducibility. Analyses of samples containing water-soluble organic matter and low concentrations of nitrate tend to give erratic values. These erratic values have been attributed to: reduction of nitrate by organic matter in the presence of strong acids; considerable charring, which contributes color that is measured as nitrate in some procedures; and organic anions, which are often co-extracted and interfere in the determination of nitrate.

In oxidation methods, e.g., using brucine, the color developed does not always obey Beer's law, and continual calibration of the standard curve is necessary (Ibid).

Interference from nitrite, chloride, and carbohydrate has been reported (ibid).

Reduction of nitrate to nitrite can be accomplished by the use of various reagents, such as copper, zinc, hydrazine sulfate, cadmium powder, and spongy cadmium (Usher and Telling, 1975). The reducing agents are not sufficiently specific and most of them give either incomplete reduction or further reduction of nitrite to ammonia. Since nitrite is ultimately determined, both defects tend to give low results. Likewise, reduction of nitrate to ammonia with zinc or any one of a number of alloys or amalgams tends to be incomplete at the concentration levels common in water samples.

Because of these analytical problems determinations of nitrate in water samples should not be regarded as valid to better than 10% or 1 mg/liter. If suitable methods for the elimination of background interference are developed, then the direct ultraviolet spectrophotometric method may provide greater precision, accuracy and sensitivity.

In contrast to the determination of nitrate that of nitrite is highly sensitive to 1 μg/liter and generally convenient and accurate. The methods are based on reaction with a primary aromatic amine in acid solution to form a diazonium salt followed by coupling with a second phenol or aromatic amine to give an intensely colored azo dye.

Conclusions and Recommendations. Nitrate in water at concentrations less than a thousand milligrams per liter is not of serious concern as a direct toxicant. It is a health hazard because of its conversion to nitrite. Nitrite is directly toxic by reaction with hemoglobin to form methemoglobin and cause methemoglobinemia. It also reacts readily under appropriate conditions with secondary amines and similar nitrogenous compounds to form N-nitroso compounds which are strongly and widely carcinogenic as a class.

Epidemiological evidence on the occurrence of methemoglobinemia in infants tends to confirm a value near 10 mg/liter nitrate as nitrogen as a maximum concentration level for water with no observed adverse health effects, but there is little margin of safety in this value.

The highly sporadic incidence of methemoglobinemia when drinking water that contains much greater concentration of nitrate is used suggests, however, that other factors than nitrate intake are important in connection with development of the disease. For example, no cases of clinical methemoglobinemia could be found in two recent studies of communities in Southern

California and central Illinois where the water supplies were on occasion found to contain as much as 20 mg/liter of nitrate as N (Shearer, *et al.*, 1972; Winton, *et al.*, 1970). More research is needed on the metabolism of nitrate and on factors that affect the rate of extent of reduction to nitrite, as well as on those that influence subsequent reaction of nitrite to form methemoglobin.

Each link in the chain of reactions from nitrate to N-nitroso compound has been shown to occur in some conditions in man or other animals. The extent of operation of the overall reaction chain in man has not been shown however, nor is there knowledge of the ways in which other environmental or internal factors may affect potential formation of N-nitroso compounds. In particular, relative effects of salivary nitrate as compared with nitrate in imbibed water have not been elucidated. There is thus little scientific basis to support conclusions on the safety of any concentration of nitrate in water with regard to carcinogenic potential.

Sulfate

Occurrence. Sulfate is found almost universally in natural waters in concentrations ranging from a few tenths of a milligram/liter up to several thousand milligrams/liter. It occurs frequently in rainfall, particularly from airmasses that have encountered metropolitan areas, sometimes at concentrations greater than 10 mg/liter.

One of the most important terrestrial sources is evaporite sediment, from which magnesium, sodium and especially calcium sulfate may be leached. Metallic sulfides, such as iron pyrites, occur in both igneous and sedimentary rocks; they are oxidized to sulfate by moist oxygen during weathering processes. Some sulfate is formed during oxidative decay of organic matter.

Sulfate may also enter water courses through waste discharges. Household wastes, including detergents, add ten or more milligrams/liter of sulfate to sewage. Tanneries, steel mills, sulfate-pulp mills and textile plants are all important industrial sources of sulfate.

In the 1970 survey of drinking water supplies the range of sulfate concentrations for the 969 samples was from less than 1 mg/liter up to 770 mg/liter with a median of 46 mg/liter. Twenty-five supplies, about 3% of those tested, showed sulfate in excess of the maximum recommended value in the 1962 U.S. Public Health Service Drinking Water Standards (250 mg/liter). Similar results were obtained in the Interstate Water Carrier Analyses of 1975 (EPA, 1975). Of 625 supplies analyzed, 21 or 3.4% were found to contain sulfate greater than 250 mg/liter, the greatest concentration being 970 mg/liter.

Once sulfate has been dissolved in water, it becomes a permanent solute except when it is anaerobically reduced to sulfide and precipitated in sediments, released to the atmosphere as H_2S, or incorporated in living organic matter. Most inorganic sulfates are quite soluble except for the lead and barium salts. Sulfate is not removed from water by any of the common treatment processes. Desalination techniques such as ion exchange, reverse osmosis or membrane electrodialysis must be employed.

Health Aspects. The major observed health effect of sulfate is its laxative action. Peterson (1951) observed that when the laxative doses of Glauber's salt, Na_2SO_4, and Epsom salt, $MgSO_4$, were translated into water concentrations based on a 2-liter daily supply, the laxative concentrations should be 300 mg/liter for Na_2SO_4 and 390 mg/liter for $MgSO_4$.

Results of a survey on the reactions of water consumers by the North Dakota State Department of Health (Peterson, 1951) indicated that there was a perceived laxative effect at 750 mg/liter, but not at 600 mg/liter or less. The presence of Mg^{++} at a concentration about equivalent to that of sulfate made the laxative effect manifest at lesser sulfate concentrations.

A more detailed analysis of the data by Moore (1952) led to the conclusion that most people experienced a laxative effect when sulfate plus magnesium exceeded 1000 mg/liter. Moore's tabulation of the data is shown in Table V-25.

TABLE V-25

WATER QUALITY OF WELLS AS RELATED TO PRESENCE OR ABSENCE OF LAXATIVE EFFECTS

Substance In Water	Concentration mg/liter	No. Wells	Effects Yes	Effects No	Laxative Not Stated	Number Of "Yes" Answers*
Magnesium plus sulfate	0- 200	51	8	30	8	21
	200- 500	45	7	27	11	21
	500-1,000	56	11	28	17	28
	1,000-1,500	36	18	10	8	64
	1,500-2,000	14	6	4	4	60
	2,000-3,000	21	13	3	5	81
	over 3,000	14	5	1	8	83
Sulfate	0- 200	56	10	36	10	22
	200- 500	47	9	28	10	24
	500-1,000	56	13	26	17	33
	1,000-1,500	34	16	10	8	62
	1,500-2,000	16	9	4	3	69
	2,000-3,000	20	9	3	8	75
	over 3,000	8	3	0	5	100

* Based only on total of "yes" and "no" answers. It is probable that a large proportion of the wells for which no statements were made were not regularly used as water supplies.

(From Moore, 1952)

An *et al.*, (1967) reported that concentrations of sulfate in water between 500 and 1000 mg/liter caused slight, but significant, decrease in acidity of gastric juice. Also, persons living at Shumilova Settlement experienced diarrhea and taste deterioration when the concentration of sulfate increased from 571-1,235 mg/liter. Korotchenok (1946) however, reported that there were no acute toxic effects noted in Western Turkmenia (U.S.S.R.) from the consumption of water with as much as 1,295 mg/liter of sulfate.

Macfayden (1953) reported on one village in British Somaliland that was using a water containing sulfate at a concentration about 4,400 mg/liter.

Studies by Digesti and Weeth (1973) gave results indicating that growing cattle can tolerate water containing sulfate at 2,500 mg/liter without ill effect. They concluded, moreover, that this value was nearly the maximum safe concentration.

The taste threshold for sulfate in water and in has been reported to lie between 300 and 900 mg/liter (Whipple, 1907; Lockhart *et al.*, 1955). Table V-26 shows a summary of the data.

TABLE V-26

INFLUENCE OF SULFATES
TASTE OF WATER AND COFFEE

Threshold concentration mg/liter

	Median		Range		Average	
Compound	Salt	Anion	Salt	Anion	Salt	Anion
$Na2SO4$	350	327	250-550	169-372	--	--
$CaSO4$	525	340	250-900	177-635	--	--
$MgSO4$	525	419	400-600	320-479	500	400

(from Lockhart *et al.*, 1955)

The 1962 Drinking Water Standards of the U.S. Public Health Service recommended that sulfate in water should not exceed 250 mg/liter, except when no more suitable supplies are or can be made available. The World Health Organization, in its European Standards For Drinking Water set a sulfate limit of 250 mg/liter (WHO, 1970).

Other Aspects. Ingleson *et al.* (1949) state that sulfate appears not to increase the corrosion of brass fittings in domestic water systems. Holl (1935) found that concentrations of sulfate less than 200 mg/liter do not increase the plumbosolvency of water.

Kellam (1933) has reported that sulfate at concentration less than 25 mg/liter has little effect on the corrosiveness of water toward concrete, but Hammerton (1945) claims that concentrations greater than 1000 mg/liter cause rapid corrosive attack.

Great concentrations of sulfate may be toxic to plants. According to Schofield (1936) water containing more than 960 mg/liter of sulfate is unsuitable for irrigation.

Analysis. The preferred standard technique for determination of sulfate is precipitation of barium sulfate followed by ignition of the collected precipitate. Results for concentrations in the range of 100 mg/liter have shown standard deviations near 5%; the sensitivity is about 1 mg/liter (A.P.H.A., 1976).

Routine determinations can be performed more conveniently with only slight decrease in precision by collecting and drying the barium sulfate on a fritted glass of membrane filter, rather than igniting it.

Turbidimetric measurement of precipitated barium sulfate is rapid and particularly suitable for concentrations of sulfate less than 100 mg/liter. The limiting sensitivity remains nearly 1 mg/liter, however.

Conclusions and Recommendations. No adverse health effects have been noted for concentrations of sulfate in water less than about 500 mg/liter. The only observed physiological effect at greater concentrations to more than 1000 mg/liter has been the induction of diarrhea.

The taste threshold for sulfate in water lies between 300 and 400 mg/liter for most persons, but some individuals are able to detect as little as 200 mg/liter.

Summary of Other Inorganic Constituents

Arsenic. Arsenic is widely distributed in the waters of the United States but generally in low concentration. In 728 samples of surface water, the concentration of arsenic ranged from less than 10 μg to a maximum of 1,200 μg/liter. The median value for arsenic in river waters was less than 10 μg/liter. High levels of arsenic in well waters have also been reported; occasionally exceeding 1 mg/liter.

Waters may be contaminated with many different forms of arsenic, each of which has different toxicological properties. Arsenic occurs in many different forms which vary in their solubility and other physical and chemical properties. Those of most concern are water soluble compounds.

Arsenic is found in pork, poultry and shellfish; the last may contain up to 170 ppm. The daily median intake of arsenic in the U.S. from all sources has been estimated to be 137 - 330 micrograms.

A major problem in understanding the metabolism and toxicity of arsenic has been the difficulty in finding a suitable animal model.

The relative toxicity of different forms of arsenic can be explained, in part, by the fact that the more toxic trivalent compounds, are retained in the tissues in greater amounts and are excreted more slowly than the less toxic.

Arsenic affects tissues that are rich in oxidative systems primarily the alimentary tract, kidneys, liver, lungs and epidermis. It is very damaging to capillaries and this results in hemorrhage into the gastrointestinal tract, sloughing of mucosal epithelium, renal tubular degeneration, hepatic fatty changes and necrosis.

The major characteristics of acute arsenic poisoning in humans are profound gastrointestinal damage and cardiac abnormalities. Subacute exposure results in vomiting, diarrhea, conjunctivitis, rhinitis, laryngitis, bronchitis, skin eruptions, neurologic signs and symptoms, muscle tenderness, and transverse white ridge on the finger nails (Mees lines).

Chronic arsenic toxicosis has not been encountered to any significant extent in animals; effects in man include cancer of the skin and lungs. In most human exposure, concomitant exposures to other agents confound interpretation of observations.

Although there appears to be some doubt regarding the carcinogenicity of arsenic compounds, there is epidemiological evidence that cutaneous lesions (leukoderma, melanoderma, hyperkeratosis, squamous-cell carcinoma) are associated with drinking water with higher than normal arsenic concentration. The city of Antofogosta, Chile is a remarkable example of cause and effect. A city of 100,000 population drinking for decades a water containing a weighted average arsenic of 598 μg/liter resulted in an incidence of cutaneous skin lesions of 313/100,000 per year. After the water treatment plant was completed, which reduced the arsenic level to 80 μg/liter, the incidence of cutaneous lesions dropped to 19/100,000 per year. This finding suggests that even 80 μg/liter exceeds the acceptable level for a public water supply.

Animal studies have failed to demonstrate carcinogenicity for arsenic compounds; mutagenicity and teratogenicity studies have yielded variable results.

In conclusion, there is some epidemiological evidence that high concentrations of arsenic in drinking water are associated with skin cancer. When the level was reduced by water treatment to 80 μg/liter, the incidence was reduced but still detectable. The existence of other cocarcinogens in these water supplies has not been extensively studied. If the time factors for the development of cancer are shown to be reasonable, then the current interim standard of 50 μg/liter may not provide an adequate margin of safety.

Research Needs

1. Improvement of analytical techniques and methodology for better adaptability to water and foods; definition of chemical form is required.

2. Epidemiologic and analytical studies to determine extent of the various forms of arsenic at low concentrations in the environment and their relation to disease patterns.

3. Development of a suitable animal model for a study of low level long term studies.

4. Intensive studies into the metabolism of arsenic in mammalian systems.

5. Studies about interaction of arsenic, with other trace elements in the environment (Se, Cu, Zn, etc.).

Selenium. Selenium is found in water principally as a result of leaching from rocks and soils which are high in selenium content. Most toxicity from selenium is a result of drinking water from wells drilled through seleniferous shales rich in soluble selenium. There is an insufficient amount of selenium in water alone to provide even the nutrient requirements of most animals, but concentrations vary in different places. Analyses of 535 samples of water from major U.S. watersheds indicated that over a four-year period only two samples contained selenium at more than 10 µg/liter of water, the current U.S. interim drinking water standard.

In another study, a maximum of 10 and a mean of 8 µg/liter in 194 public finished water supplies in the U.S. has been reported. In Germany and in Australia, village water supplies have been reported to contain from 1 - 5.3 µg/liter. Shallow or deep wells contain varying concentrations of selenium. For example, deep wells in Wyoming may contain only a few micrograms per liter while other wells contain enough selenium to be poisonous to man and livestock; some of these have been associated with the loss of hair and nails in children. Foods from nonseleniferous areas contribute very little to the overall dietary intake of selenium. Eggs and milk, fish, various types of meat, poultry, coffee, and tea all vary somewhat in their selenium content, but in general contribute minimally to the dietary intake.

Inorganic and organic forms of selenium are readily absorbed from the gastrointestinal tract of animals. Selenite is absorbed more rapidly by monogastric animals compared to ruminant animals, perhaps due to bacterial reduction of selenite to elemental selenium or other insoluble forms in the ruminant gastrointestinal tract.

Selenite and selenate are distributed largely to the liver, kidneys, muscle mass, gastrointestinal tract and blood. Chronic administration of selenium results in increased concentration in the testes. The principal route of excretion of selenium is via the urine.

At higher exposure levels, selenium is incorporated into molecules normally served by sulfur; it is methylated by mammalian tissues in an apparent detoxication process. Selenite and selenate are metabolized to trimethyl-selenonium ion which is the principal excretory product for selenium in urine.

The toxicity of selenium can be altered extensively by interactions with sulfate, methionine, cystine, mercury, lead, zinc, cadmium, copper, arsenic, and vitamin E, but little is known about these interactions.

Selenium is essential for domestic animals but the margin of safety is relatively narrow. A low level of selenium is essential to prevent myopathies, liver injury and congenital abnormalities in domestic and laboratory animals and poultry and there is little reason to believe that humans differ appreciably in this regard; however, data are lacking. High dietary selenium is toxic to animals and a defined set of signs and lesions have been established for acute, and subchronic exposure to toxic concentrations in several species. Chronic, long-term studies have been limited primarily to feeding studies in the rat and those have involved relatively high concentrations of the element.

With the exception of a limited number of reports of acute exposures to toxic levels under industrial circumstances, or other accidental exposure, an indication for health effects on human populations must be extrapolated from animal data. Inhalation causes acute respiratory distress, and skin exposure causes severe local irritation and dermatitis. The severity of response will depend on the chemical form of selenium.

In animals, acute exposure to selenium causes respiratory distress, diarrhea, pulmonary edma, hemorrhage, liver and renal necrosis. Chronic exposure results in death from gastroenteritis, myocardial damage, hydrothorax, pulmonary edema, renal and liver damage. Sodium selenite is toxic to rats at concentrations of 6-9 mg/liter in drinking water; concentrations of 1 mg/liter are without observed toxic effect.

Chronic exposure of humans to selenium by inhalation or by ingestion results in central nervous system and gastrointestinal disturbances, and dermatitis.

The high selenium content of diet and water in areas of seleniferous soils have been associated with "alkali disease" in cattle. Human populations living in these areas are not similarily affected. This is believed to be due to the wide geographical sources of food consumed and the loss of selenium during processing. Where feed is low in selenium, water contaning 400-500 μg/liter is too low to cause poisoning in the cattle. For livestock water the maximum recommended concentration is 50 μg/liter.

The only documentation of human toxicity from drinking water involved a family consuming well water which contained selenium at 9,000 μg/liter. In 1942, the USPHS drinking water standard listed selenium for the first time along with fluoride and arsenic. The level set was 50 μg/liter and easily met by public water supplies.

The interim standard of 10 μg/liter was recommended in 1962 because of evidence that selenium was carcinogenic in animal studies. The current literature review of animal studies does not support this contention nor is there any epidemiological evidence implicating a higher than normal cancer incidence among those having higher than normal daily intake of selenium.

The established requirement for selenium in most animal species indicates a need for more data on potential or real deficits or excesses in human populations. The concentration of selenium in waters of the United States varies widely and currently there is no evidence to suggest a problem. The totally of evidence indicates that there is greater overall potential for selenium deficiency than for selenium toxicity with current intake levels of selenium. The maximum no-observed-adverse health effect level for selenium in water is not less than 100 μg/liter and appears to be as great as 500 μg/liter. A concentration of 20 μg/liter just barely provides a minimum nutritional amount of selenium with a consumption of 2 liters a day.

In conclusion, there is evidence that selenium may be an essential trace element for humans. The current interim standard of 0.01 μg/liter was established because there was some concern that selenium was a carcinogen. This claim cannot be supported by a review of the current literature. To this end these effects must be investigated and the current interim standard re-evaluated.

The paucity of definitive data on selenium and human health requires a number of research approaches to elucidate the role of selenium in the mammalian system. The following research needs are suggested:

1. Techniques need to be developed for more rapid, accurate and reproducible analytical methods which will permit both qualitative and quantitative assays of chemical forms, oxidation state, and solubility in water.
2. Improved systems for monitoring selenium in the environment (water, air food).
3. Research to define molecular transformations in the mammalian system.
4. Programs to study interactions between selenium, mercury, cadmium, arsenic and other trace elements and heavy metals in the biosphere and in animal organisms.
5. A determination of natural and industrial emissions and cycling of selenium in the environment.
6. Effects in animal system of long-term, low levels of selenium singly and in combination with other trace elements in the environment.

7. baseline data on selenium levels in humans in health and disease.

8. Effects of deficiency or an excess of selenium on the development of animal tumors.

9. Determine whether some segments of the human population of the United States require additional selenium for optimum health.

Fluoride. Fluoride is found widely in water supplies, but the concentration is usually not great enough to be undesirable. The maximum concentration found for the 969 supplies studies in the 1969 Community Water Supply Survey was 4.4 mg/liter. Most supplies not fluoridated had fluoride concentrations less than 0.3 mg/liter.

A more extensive survey by the Dental Health Division of the U.S. Public Health Service showed more than 3,600 communities with a population of 8 million people had water supplies with more than 0.7 mg/liter of naturally occurring fluoride. Most of these communities are in Arizona, Colorado, Illinois, Iowa, New Mexico, Ohio, Oklahoma, South Dakota and Texas. Of these, 524 communities representing one million people had fluoride concentrations over 2 mg/liter. objectionable mottling even with high temperatures.

Small amounts of fluoride, of the order of 1 mg/liter, depending on the environmental temperatue, in ingested water and beverages, are generally conceded to have a beneficial effect on dental caries, particularly among children. This review did not systematically review the evidence for the beneficial effects of fluoride.

Two forms of chronic toxic effects are recognized generally as being caused by excess intake of fluoride over long periods of time. These are mottling of tooth enamel or dental fluorosis, and skeletal fluorosis. In both cases, it is necessary to consider the severity since the very mild forms are considered beneficial by some. The most sensitive of these effects is the mottling of tooth enamel, which, depending on the temperature, may occur to an objectionable degree with fluoride concentrations in drinking water of only 1.5-2.0 mg/liter. Apparently there has been little systematic investigation of the degree to which consumers of drinking water with several mg/liter of fluoride regard the resultant mottling as an adverse health effect.

Skeletal fluorosis has been observed with use of water containing more than 3 mg/liter. It now appears that long-term consumption of water containing fluoride in excess of 1 mg/liter runs into a fair probability of objectionable dental mottling and increased bone density in patients with long-standing renal disease or polydipsia. Increased bone density, however, has often been regarded as a beneficial rather than an adverse effect.

Intake of fluoride for long periods in amounts greater than 20-40 mg/day may result in crippling skeletal fluorosis.

Other reported adverse health effects of intake of milligram per liter levels of fluoride in drinking water, including mongolism, cancer mortality, mutagenic or birth effects and sensitivity have either been unconfirmed or found lacking in substance. There is also no evidence that there is any difference between the effects of naturally occurring or intentionally added fluoride.

Epidemiological studies where the water is naturally high in fluoride have found no adverse effects except in rare cases, until the concentration is many times that recommended for added fluoride. Controlled studies with fluoridiation at the 1 mg/liter level have reported no instances of adverse effects. Available evidence does not suggest that fluoridation has increased or decreased cancer mortality rates; the margin of error is very low, approaching 2 per 100,000. This is the theoretical effect that could have been missed with present statistical techniques.

Additional studies of mottling and skeletal fluorosis need to be done in communities with several mg/liter fluorine in their water supplies to ascertain whether the no-adverse-health effect level for fluoride is greater or less than 1 mg/liter. In addition sociological studies are needed to ascertain the extent to which dental mottling is regarded as an adverse effect.

Sodium. Sodium ion is an ubiquitous constituent of natural waters. It is derived geologically from the leaching of surface and underground deposits of salts such as sodium chloride, from the decomposition of sodium aluminum silicates and similar minerals, from the incorporation of evaporated ocean spray particles into rainfall and from the intrusion of sea water into fresh water aquifers. The sodium chloride used as a deicing agent on roads enters water supplies in runoff from both roads and storage depots. This added sodium chloride amounting to 9 million tons in 1970 is distributed throughout the snow belt of the northern U.S. and is most heavily concentrated around metropolitan areas.

A survey of 2,100 supplies, covering approximately 50% of the population of the U.S., was carried out in 1963-1966. The distribution of sodium ion found in this survey ranged from 0.4-1900 mg/liter. Some 42% of the supplies had sodium ion concentrations in excess of 20 mg/liter and nearly 5% had concentrations greater than 250 mg/liter.

Few studies of habitual sodium ion intake for healthy adults in the U.S. have been reported. Such data as have been reported are based on measurement of sodium excretion in 12 or 24 hour urine collections. Wide variations occur among individuals and in the same individual from day to day. One study reported mean 24 hour excretion of 4,100 mg with a range from 1,600- 600 mg in 71 working adult males in New York. Another reported a mean sodium excretion near 2800 per 24 hours in 171 black women ranging in age from 35 to 44 years. A recent estimate for infants is 69-92 mg/kg/day.

Sodium chloride is added to many foods during processing. Additional sodium chloride is often added during cooking, and again at the table. None of this is essential, for habitual intake of sodium bears no relationship to physiological need. Healthy individuals have been shown to maintain sodium balance on a sodium intake of less than 2000 mg/24 hours while sweating 9 liters/day. A variety of preindustrial societies, in widely divergent habitats (tropical jungle, desert, arctic etc.) subsist for generations on sodium intake less than 1000 mg/day and show no evidence of sodium deprivation. Requirements for sodium in growing infants and children are estimated at less than 200 mg/day.

It thus appears that habitual intake of sodium in adults in the United States often exceeds body need by tenfold or more. Evidence that this excessive intake may have harmful consequences is summarized in the detailed report.

Specification of a "no-observed-adverse-health-effect" level in water for a substance like sodium, for which the effect is associated with total dietary intake and for which usual food intake is already greater than a desirable level, is impossible.

Since adult fluid intake averages 1.5-3 liters/day, sodium intake from drinking water represents less than 10% of the habitual total intake of 3000-4000 mg as long as the sodium content of the water does not exceed 200 mg/liter. Adverse health effects may be anticipated with sodium concentrations in water greater than 20 mg/liter only for that special risk group restricted to total sodium intake of 500 mg/day, because intake from food cannot be reduced feasibly to less than 440 mg/day. For this group, whose diets must be medically supervised in a careful manner, knowledge of the sodium ion concentration of the drinking water permits prescription of bottled water low in sodium when necessary.

A larger proportion of the population, about 3%, is on sodium-restricted diets calling for sodium intake of less than 2000 mg/day. The fraction of this that can be allocated to water varies, depending on medical judgment for individual instances. Knowledge of the sodium ion content of the water supply and maintenance of it at the lowest practicable concentration is clearly helpful in arranging diets with suitable sodium intake.

in many diets allowance is made for water to contain 100 mg/liter of sodium.

It appears that at least 40% of the total population would benefit if total sodium ion intake were maintained at not greater than 2000 mg/day. With sodium ion concentration in the water supply not more than 100 mg/liter, the contribution of water to the desired total intake of sodium would be 10% or less for a daily consumption of two liters.

Nitrate. All sources of combined nitrogen must be regarded as potential sources of nitrate, for there is a tendency for all nitrogenous materials in natural waters to be converted into nitrate. Major point sources of combined nitrogen in water are municipal and industrial wastewaters, refuse dumps, animal feed lots and septic tanks. Diffuse sources include runoff or leachate from manured or fertilized agricultural lands, urban drainage and biochemical nitrogen fixation. Some tenths of a milligram/liter of combined nitrogen occurs in rainfall from solution of atmospheric ammonia and oxides of nitrogen.

In the Community Water Supply Survey of the Bureau of Water Hygiene in 1969, the range of nitrate concentrations found was 0.0-127 mg/liter. Nineteen systems, about 3% of those examined for nitrate, had concentrations in excess of the then recommended limit of 45 mg/liter.

Ordinarily, the major human intake of nitrate is from food rather than from water. The mean food intake in the United States has been estimated to be nearly 100 mg/day, most of it coming from vegetables such as spinach, lettuce and root vegetables, which may contain several thousand parts per million of nitrate.

Nitrate is secreted in the saliva, the mean value being about 40 mg/day, of which about 10 mg/day is reduced to nitrite and found in that form. These quantities, although internally derived, also represent inputs to the gastric system.

Two health hazards are related to the consumption of water containing large concentrations of nitrate (or nitrite): induction of methemoglobinemia, particularly in infants, and possible formation of carcinogenic nitrosamines.

Acute toxicity of nitrate occurs as a result of reduction to nitrite, a process that can occur under specific conditions in the stomach, as well as in the saliva. Nitrite acts in the blood to oxidize the hemoglobin to methemoglobin, which does not perform as an oxygen carrier to the tissues. Consequently, anoxia and death may ensue.

Healthy adults are reported to be able to consume large quantities of nitrate in drinking water with relatively little, if any, effects. Acute nitrate toxicity is almost always seen in infants rather than adults. This increased susceptibility of infants has been attributed to high intake per unit weight, to the presence of nitrate-reducing bacteria in the upper gastrointestinal tract, to the condition of the mucosa and to greater ease of oxidation of fetal hemoglobin.

Assessment of maximum nitrate levels in water exhibiting no adverse health effects has been based principally on a study of known cases of methemoglobinemia. No cases of methemoglobinemia have been reported when the water contained less than 10 mg/liter nitrate as nitrogen. Later, a small percentage of cases were found in which the nitrate concentration of the drinking water was somewhat less than 45 mg/liter. Only one U.S. case has been associated with a public water supply regardless of nitrate content.

Studies supplementary to the previous ones in which levels of methemoglobin in the blood of infants were related to concentrations of nitrate in the water being fed showed detectable enhancement of methemoglobin levels in infants being supplied with water containing nitrate as nitrogen only slightly in excess of 10 mg/liter.

It can be concluded that, from the view-point of induction of methemoglobinemia, the maximum concentration of nitrate in water exhibiting no significant adverse health effects is close to the interim standard of 10 mg/liter as nitrogen. However, there appears to be little margin of safety for some infants with the standard at this concentration.

The other health hazard proposed for nitrate in water, that it may act as a pro-carcinogen, is more speculative. A series of reactions is involved by which it is proposed that nitrate in water may be converted to N-nitroso compounds that are carcinogenic agents. The steps in the reaction sequence are:

1. reduction of nitrate to nitrite.
2. reaction of nitrite with secondary amines or amides in food or water to form N-nitroso compounds.
3. carcinogenic reaction of N-nitroso compound.

Reaction of nitrites and secondary amines or amides to form N-nitroso compounds occurs readily in acidic solution and particularly at the normal pH range of 1 to 5 characteristic of gastric contents after a meal.

However, the relation of nitrate concentrations in water supplies to the presence of nitrite in the digestive tract is much more problematic. The major source of nitrite to the stomach, at least for healthy individuals, is the saliva, normally containing 6-15 mg/liter of nitrite. Little reduction of nitrate to nitrite occurs in the human stomach unless the gastric pH is greater than 4.6. Thus the pH condition for formation of nitrite is quite different from the pH range for ready formation of N-nitroso compounds, pH 3.5 or less.

Epidemiologically, correlations have been shown to occur between incidence of gastric cancer and concentration of nitrate in the drinking water. An unusually high incidence of stomach cancer in certain mountainous areas of Colombia is associated with high concentration of nitrate in the drinking water.

The findings, however, are preliminary and only suggestive. They provide no firm evidence of a causal link between incidence of cancer and high intake of nitrate. They do indicate a need for caution in assessment of lack of adverse health effects even at the 10 mg/liter concentration level for nitrate as nitrogen and a need for continued intensive study on the metabolism and effects of nitrate in man.

In conclusion, epidemiological evidence on the occurrence of methemoglobinemia in infants tends to confirm a value near 10 mg/liter nitrate as nitrogen as a maximum concentration level for water with no observed adverse health effects, but there is little margin of safety in this value. There is little scientific basis to support conclusions on the hazard of any concentration of nitrate in water with regard to carcinogenic potential.

Sulfate. No adverse health effects have been noted for concentrations of sulfate in water less than about 500 mg/liter. The only observed physiological effect at concentrations greater than 1000 mg/liter has been the induction of diarrhea.

The taste threshold for sulfate in water lies between 300 and 400 mg/liter for most persons, but some individuals are able to detect as little as 200 mg/liter.

WATER HARDNESS AND HEALTH

Introduction

The principal focus of Drinking Water And Health is on possible adverse health effects from contaminants in drinking water. However, certain inorganic or mineral constituents of drinking water that, by usual definition, are not considered to be "contaminants" have been recently reported to be of public health importance. These are constituents that are associated with the level of "hardness" of the water and which occur naturally or which are picked up from water treatment or distribution systems.

Hardness may be defined as the sum of the polyvalent cations present in water. The most common such cations are calcium and magnesium. Hardness usually is expressed in terms of the equivalent quantity of calcium carbonate ($CaCO_3$). There are no distinctly defined levels for what constitutes a hard or a soft water supply. Generally, water with less than 75 mg/liter (ppm) of $CaCO_3$ is considered soft and above this concentration as hard.

There has been a great deal of interest in the relationship between the hardness of drinking water and morbidity and mortality since the studies, almost 20 years ago, of Kobayashi in Japan (1957) and Schroeder (1960) in the United States. Subsequently, numerous other studies have been carried out throughout the world which indicate some water factor(s) are statistically correlated with pathologic effects, particularly various cardiovascular diseases. As a result, a voluminous body of literature on these studies has developed and the problem has been the subject of several comprehensive reviews. The Subcommittee on Morbidity and Mortality in preparing this report has relied heavily on several of these reviews, notably those by Craun and McCabe (1975), Hayden (1976), Neri *et al.*, (1974), Sauer (1974), Sharrett and Feinleib (1975), Schroeder and Kraemer (1974), and Winton and McCabe (1970). These reviews have been abstracted and summarised rather than reprinting the same material or attempting another review.

It should be noted, also, that the World Health Organization and the International Atomic Energy Agency consider that there is sufficient evidence for the involvement of trace elements in the pathogenesis of cardiovascular diseases to warrant international collaborative studies on the problem (IAEA, 1973; WHO, 1973). The possible causal association between water hardness and cardiovascular disease has been recognised in Great Britain to be of enough potential public health importance to have resulted in official governmental expert review of the problem (MRC, 1970; COMA, 1974).

More than 50 studies in nine countries have been carried out on possible relationship of water hardness and health. Most of the investigations were in the United Kingdom, United States, and Canada; they reveal a consistent trend of significant statistical associations between the hardness characteristics of drinking water and the incidence of cardiovascular problems (heart disease, hypertension and stroke) and, to a lesser extent, other diseases. Generally, reports have shown an inverse correlation between the the incidence of cardiovascular disease and the amount of hardness of drinking water, or conversely, a positive correlation with the degree of softness. Studies in the United States and Canada have shown that age-adjusted cardiovascular mortality rates among populations using very soft water may be as much as 15-20% higher than among populations using hard water. The differential reported for the United Kingdom may be as high as 40%.

Cardiovascular diseases are the leading cause of death in the United States where they account for more than 50% of all causes of death, or roughly one million deaths each year and death rates from coronary heart disease have been steadily increasing over the past few decades.

It is evident, therefore, that if water factors are ultimately proven to be involved causally in the pathogenesis of cardiovascular disease, then we are confronted with a major public health problem and current water treatment practices will have to be greatly modified.

The credibility of these water factor studies depend more on the consistent trend of the findings than their biological plausibility or the size of the correlation coefficients or the actual significance levels. However, there is some scientific justification for the biological plausibility of these associations. There has been increasing evidence that certain trace elements play an important role in a number of biological processes through their action as activators or inhibitors of enzymatic reactions, by competing with other elements and proteins for binding sites, by influencing the permeability of cell membranes, or through other mechanisms. It is assumed that these elements can also directly or indirectly exert an action on cardiac cells, the blood vessel walls, on blood pressure, or other systems related to cardiovascular function, such as lipid and carbohydrate metabolism. It is assumed further that water quality can affect man's trace element or mineral balance and, consequently, cardiovascular function.

As previously noted, the preponderance of reported evidence indicates statistically significant correlations between some drinking water factor(s) and the incidence of cardiovascular diseases resulting in a general impression that inorganic substances in water may be causally implicated. It must be emphasized, however, that there is considerable disagreement among various investigators concerning the magnitude or even the existence of a "water factor" risk; the identity of the water factor(s); the mode of action, and the specific pathologic effects.

Theories on Risk Factors

Several hypotheses have been offered on how components of drinking water may affect cardiovascular function and disease; these generally fall into one of the following classes:

1. that one or more of the principal "bulk" constituents of hardness in tap water are protective.
2. that one or more of the trace elements that tend to be present in hard water are protective.
3. that harmful metals are present in soft water, possibly having been picked up by leaching from the distribution system.
4. that other factors are involved. Each class of hypotheses is briefly reviewed below.

1. Protective effect from bulk constituents of hard water. Hardness is not a specific constitutent of water but a complex and variable mixture of cations and anions. Several investigators have attributed the disease protective effect of hard water to the presence of calcium and magnesium, which are the principal cations found in hard water. Calcium, magnesium and hardness generally correlate well with one another. In a few studies, however, it was possible to discriminate between the two elements and treat them as separate variables. When calcium and magnesium are separately correlated with cardiovascular disease rates, calcium appears to correlate with greater significance in the United Kingdom, whereas in the United States the correlations are about equally strong for calcium and magnesium.

There is a limited amount of evidence to explain the possible mechanism whereby calcium and/or magnesium may play a role in protection against cardiovascular diseases. Experimentally, a moderate increase of calcium in the diet results in lower levels of circulating and organ cholesterol; this is speculated as a possible factor in the association noted between water hardness and cardiovascular diseases. Magnesium is theorized to protect against lipid deposits in arteries and also may have some anti-coagulant properties that could protect against cardiovascular diseases by inhibiting blood clot formation. Also, there is evidence to indicate that there may be higher concentrations of calcium and magnesium in certain tissues among residents of hard water areas as compared to soft water areas.

2. Protective action of trace elements in hard water. There is a paucity of systematic data concerning the concentrations of trace elements as a correlate of hardness of water and cardiovascular disease rates. From a limited number of studies that have been carried out, if hard water contains protective beneficial elements (other than calcium and magnesium), vanadium, lithium, and possibly manganese and chromium, emerge as candidates.

Lithium and vanadium have been reported to be negatively correlated with cardiovascular mortality. These negative correlations appear to persist and remain significant even after controlling for calcium and magnesium. The biological functions of these metals are obscure. It is speculated that lithium may have a specific influence on catecholamines and coronary prone behavioral patterns. Vanadium is reported as an essential trace element in human nutrition and thought to inhibit hepatic cholesterol synthesis and reduce serum cholesterol. Increased intake of vanadium is believed therefore to reduce serum cholesterol. The mechanism is thought to be an inhibition of cholesterol synthesis, especially in young subjects.

A case is made that chromium which is positively correlated with the hardness of tap water in North America (but not in the United Kingdom) may be causally involved. Experimentally, chromium deficiency produces elevated serum glucose and cholesterol levels and increased deposition of aortic plaques. Though quantitative estimates of daily chromium requirements cannot be given yet, it is thought that the chromium level in hard water may help protect against a deficiency. Similarly, it is speculated that hard water may protect against a deficiency of manganese which also experimentally is associated with decreased glucose tolerance.

3. Harmful elements in soft water. Soft water tends to be more corrosive than hard water. As a result certain trace metals are found in higher concentrations in soft than in hard water. Several such metals have been suggested as possible intermediaries in the increased cardiovascular disease rates associated with soft water. Based on very limited data, cadmium, lead, copper, and zinc have been suspected to be possibly involved in the induction of cardiovascular disease. These metals often occur in plumbing materials and have been found to leach into soft drinking water.

There is evidence that relatively low doses of cadmium can produce hypertension in rats. It is known that the metal can accumulate in human kidneys and produce renal damage and presumably could affect blood pressure. However, direct evidence linking cadmium in water to heart disease in humans is lacking.

Several studies have shown elevated levels of blood lead occurring among persons living in homes having lead plumbing and soft water, or both. But the relationship between these elevated blood lead levels to cardiovascular disease remains unclear.

There is limited data suggesting that the intake levels of copper and zinc from soft water may adversely affect cardiovascular disease rates. However, there are conflicting data from other studies. Still other studies suggest that the discrepancies may be due to the failure to examine critically the ionic formand the intake ratios of the suspect metals from all sources, particularly the Zn:Cu and the Cd:Zn ratios as well as various other metabolic variables.

4. Other factors and confounding variables. From the above discussion, it is apparent that there is no shortage of hypotheses to explain how components of drinking water might affect cardiovascular function and disease. It is necessary to consider these hypotheses along with other factors and some confounding variables.

Several cations found predominantly in hard water are theorized to exert a beneficial effect on cardiovascular function and other cations found in soft water, to exert a detrimental effect. The question often raised is whether drinking water can provide enough of these elements to have any significant impact on the pathogenesis of cardiovascular diseases when considered in the context of the total intake of these elements through other dietary and environmental pathways. Hard or mineralized water generally would supply less than 10-15% of the total dietary intake for calcium and magnesium.

Water provides even a smaller proportion of the total intake for the various suspect trace metals with the possible exception of lead. The largest proportion of trace metal intake from water compared to food is for zinc, but even for this water provides only about 4% of its total dietary intake. For all other suspect metals drinking water provides under 4% of total intake. The concentrations of lead in certain drinking waters may contain levels exceeding 100 μg/liter of water as compared to an average adult daily dietary intake of about 300 μg.

Several investigators, however, point out that the amount of these elements provided through drinking water relative to other sources is less important than their chemical form. It is theorized that trace elements often occur in a chelated form in foods and may be less available metabolically than the ionized form that generally occurs in water. Also, the valence form of elements found in water may differ from that in foods and affect metabolic behavior.

Another possible variable is the different effect of hard and soft waters on the mineral composition of foods during cooking. It is theorized that soft water may remove a significantly higher proportion of various "protective" nutrients and elements from foods during cooking than do hard waters.

Most of the studies carried out to date correlate mortality rates with measurements made on raw rather than on finished water; the correlations were of lesser statistical significance when finished water is used.

There was considerable variation in the study design and methods among the numerous investigations reported. As previously noted, most of the studies report a statistically significant correlation between water-hardness and one or more of several cardiovascular diseases. It is not possible, however, to quantitatively compare the data from many of these studies because of the different criteria and indices used in the specification of cause of death. The case for a causal association of water factors to any specific pathologic effects is thought to be further weakened by several reports of correlations of the water factor with other causes of death, such as bronchitis, infant mortality, malignancies, cirrhosis, and other noncardiovascular causes of death. Despite the consistent trend for most of the reported studies, a few studies have shown negative or conflicting results for different age and sex groups. For example, in Holland and Sweden, hard water was correlated with decreased cardiovascular mortality among women but not men, and an opposite finding emerged from a study in New Foundland.

The strength and specificity of the correlative studies have varied depending on the sample sizes of the area and population. In general, the relationship appears stronger in larger and more populous areas.

To some extent these differences are probably due to a lack of sensitivity of correlation coefficients related statistically to the size of the sampling unit. Obviously, smaller geographical units with smaller populations would tend to have less stable death rates and consistency than larger ones so that any variable will tend to correlate less well with smaller geographical and population bases. But it should be noted that the size of the metropolitan area and population density tend to correlate well with cardiovascular disease rates independently of water quality. This is attributed to various cultural and socioeconomic factors that appear to influence cardiovascular disease mortality rates. On the other hand, less urban areas are more likely to use relatively hard ground water and, conversely, larger metropolitan areas are usually more dependent on softer surface waters. In a few studies where corrections for socioeconomic factors were attempted, the correlations with hardness of water still exist but with a reduced statistical significance. It is possible that both urbanisation and water mineralisation have an effect on cardiovascular disease rates and could be interacting or acting separately.

Several studies have shown statistically significant correlations of death rates with various geographical and climatic variables, especially rainfall, independently of water quality variables. Much more work must be done on the possible associations and interrelationships of variables such as rain, soil chemistry, and human nutrition with water quality and cardiovascular disease rates.

From this review, it is clear that there is no shortage of hypotheses related to how the components of drinking water might affect cardiovascular function and disease. Despite the large body of evidence supporting the hypotheses, there are too many confounding variables and discrepancies in the data to permit any scientifically sound conclusions as to the specific role of water factors in the pathogenesis of cardiovascular diseases.

Summary

There is a large body of scientific information that indicates certain inorganic or mineral constituents of drinking water are correlated with increased morbidity and mortality rates. These constituents by usual definition are not considered to be "contaminants" as they often are associated with the level of "hardness" of drinking water and occur naturally or are picked up from water treatment or distribution systems. Hardness is due primarily to the presence of ions of calcium and magnesium and is expressed as the equivalent quantity of calcium carbonate ($CaCO_3$). Water with less than 75 mg $CaCO_3$/liter is generally considered soft, and above 75 mg/liter as hard.

A voluminous body of literature suggests that in the United States and other developed nations, the incidence of many chronic diseases, but particularly cardiovascular diseases (heart disease, hypertension, and stroke), is associated with various water characteristics related to hardness. Most of these reports indicate an inverse correlation between the incidence of cardiovascular disease and the amount of hardness. A few reports also indicate a similar inverse correlation between the hardness of water and the risk from several noncardiovascular causes of death as well.

Several hypotheses are reported on how water factor(s) may effect health; these mostly involve either a protective action attributed to some elements found in hard water or harmful effects attributed to certain metals often found in soft water.

The theorized protective agents include calcium, magnesium, vanadium, lithium, chromium, and manganese. The suspect harmful agents include the metals cadmium, lead, copper, and zinc, all of which tend to be found in higher concentrations in soft water as a result of the relative corrosiveness of soft water.

It is evident from the review of the literature that there is considerable disagreement concerning the magnitude or even the existence of a "water factor" risk; the identify of the specific causal factor(s); the mode of action and the specific pathologic effects.

Nevertheless, the preponderance of reported evidence reflects a consistent trend of statistically significant inverse correlations between the hardness of water and the incidence of cardiovascular diseases. As a result, there is a general impression that harmful elements in soft water and/or protective elements in hard water are causally implicated in the pathogenesis of cardiovascular and possibly other chronic diseases.

The wide spectrum of alleged associated effects, the lack of consistency in theorized or reported etiologic factors, the very small quantities of suspect elements in water relative to other sources, and the discrepancies between studies raise serious questions as to whether drinking water really serves as a vehicle of causal agents, is an indicator of something broader within the environment, or represents some unexplained spurious associations. Despite these uncertainties, the body of evidence is sufficiently compelling to treat the "water story" as plausible, particularly when the number of potentially preventable deaths from cardiovascular diseases is considered. In the United States, cardiovascular diseases account for more than one-half of the approximate two million deaths occurring each year. On the assumption that water factor(s) are causally implicated, it is estimated that optimal conditioning of drinking water could reduce this annual cardiovascular disease mortality rate by as much as 15% in the United States.

In view of this potential health significance, it is essential to ascertain whether water factors are causally linked to the induction of cardiovascular or other diseases and, if so, to identify the specific factors that are involved. Much more definitive information is needed in order to identify what remedial water treatment actions, if any, can be considered.

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